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(71) Applicant *(for all designated States except US)*: INDEPENDENT PHARMACEUTICA AB [SE/SE]; Birger Jarlsgatan 14, S-114 34 Stockholm (SE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SVENSSON, Torgny [SE/SE]; Ringvägen 9, S-181 33 Lidingö (SE). JOHANSSON, Anette [SE/SE]; Lillberga, S-740 21 Järlåsa (SE).

(74) Agents: NILSSON, Brita et al.; AB Stockholms Patentbyrå, Zacco & Bruhn, P.O. Box 23101, S-104 35 Stockholm (SE).

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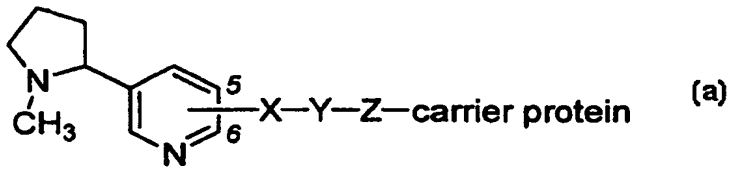


(a)

(57) Abstract

A 5- or 6-nicotinyl-linker-carrier protein compound and immunogen having formula (a) wherein X is -NH-CO- or NH- or -C≡C- or -C=C- or $-CH_2-$; Y is $-(CH_2)_k-$ or $-(CH_2)_m-C_6H_{10}-(CH_2)_n-$ or $(CH_2)_m-C_6H_4-(CH_2)_n-$ when Z is -NH-, with the proviso that X is not -NH-CO- when Y is $(CH_2)_5$ and Z is -NH-, and X is -NH-CO- or -C≡C- or -C=C- or $-CH_2-$, Y is $-(CH_2)_m-C_6H_{10}-(CH_2)_n-$ or $-(CH_2)_m-C_6H_4-(CH_2)_n-$, when Z is -CO-, and X is -C≡C- or -C=C-, Z is -CO-, when Y is $-(CH_2)_k-$, is disclosed. The compound may be used as a medicament, suitably in the form of a pharmaceutical composition. Additionally, a method of prophylactic and/or therapeutic immunological treatment of nicotine dependence from tobacco products to achieve harm reduction is described.

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# NICOTINE IMMUNOGEN

The present invention relates to a nicotine immunogen, to a 5- or 6-nicotinyl - linker -protein and its use as a medicament and a pharmaceutical composition comprising the 5- or 6-nicotinyl - linker -protein, and to a method of prophylactic and/or therapeutic immunological treatment of nicotine dependence from tobacco products to achieve harm reduction.

**Background of the invention**

Due to the vast number of tobacco users/smokers in the world who want to get rid of their dependence, there is a large market for products helping them to reach their goal or at least result in harm reduction.

One approach is to develop a vaccine/immunogen that in an individual can elicit antibodies which strongly bind to administered/inhaled nicotine and block its effect before it reaches their central nervous system. The idea is that if the individual does not experience the expected stimulating effect of nicotine administration/smoking, the interest in administering a tobacco product, such as moist snuff, or lighting a cigarette will cease (extinction/prevention).

A complementary approach is to develop an immunogen that in an individual can elicit antibodies which moderately or weakly bind to administered/inhaled nicotine and enhance/prolong its effect in their central nervous system. The idea is that if the individual experiences the expected stimulating effect of nicotine administration/-smoking during a prolonged period of time, the interest in a renewed administration of a tobacco product, such as moist snuff, or lighting a cigarette will be postponed and the medical consequences of the tobacco product consumption will be reduced.

Both of the above mentioned approaches use immunogens which in an individual induces an immunological response which leads to harm reduction.

In the prior art there are papers describing immunoassays using antibodies directed to nicotine, but such antibodies have not been suggested for any medical use (See e.g. Castro A. and Monji N. Res. Comm. Chem. Path. Pharmacol. 1986,51 , 393-404.)

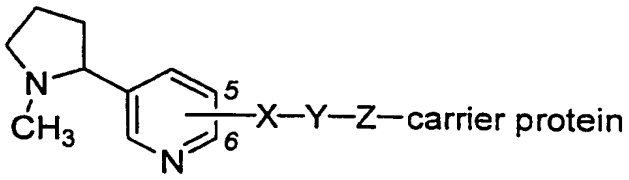
The idea of treating nicotine dependence with a vaccine is not new, since it is comprised by earlier general disclosures of manufacturing vaccines against drugs which may cause a dependence (see e.g. EP- B1- 0 496 839, exemplified by morphine; and WO 96/30049, exemplified by cocaine).

A paper disclosing active immunization to alter nicotine distribution was recently published (Hieda Y. et al, J. Pharmacol. and Exp. Therap. 1997,283, 1076-1081). The immunogen used in the experiments was (±)-6-(carboxymethyl-ureido)-nicotine linked to keyhole limpet hemocyanin.

Recently, the international patent application WO 98/14216 was published (09.04.98). This application claims a large number of hapten-carrier conjugates based on the nicotine molecule and the common structural feature of the compounds seems to be that all of the hapten molecules contain a terminal carboxylic acid group which is then conjugated to the carrier. No *in vivo* testing has been disclosed for the alleged drug abuse treatment.

**Description of the invention**

The present invention is directed to a nicotine immunogen comprising a 5- or 6-nicotinyl- linker - carrier protein having the formula




wherein

X is -NH-CO- or -NH- or -C≡C- or -C=C- or -$CH_2$- ;

Y is -$(CH_2)_k$- or -$(CH_2)_m$-$C_6H_{10}$-$(CH_2)_n$- or -$(CH_2)_m$-$C_6H_4$-$(CH_2)_n$-

wherein k = 0-20, m = 0 - 6, and n = 0 – 6, when

Z is -NH-,

with the provisio that X is not –NH-CO- when Y is $(CH_2)_5$ and Z is -NH-,

and

X is -NH-CO- or -C≡C- or -C=C- or -$CH_2$-,

Y is -$(CH_2)_m$-$C_6H_{10}$-$(CH_2)_n$- or -$(CH_2)_m$-$C_6H_4$-$(CH_2)_n$-

wherein m = 0 - 6, and n = 0 – 6, when

Z is -CO-,

and

X is -C≡C- or -C=C- ,

Z is -CO-, when

Y is -$(CH_2)_k$-

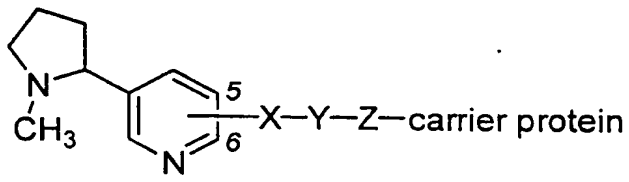
wherein k = 0 – 20.

In a preferred embodiment of the invention the 5- or 6-nicotinyl - linker - carrier proteins are selected from compounds wherein k= 1 - 8, m=0 - 3 and n=0 - 3.

Other names for the 5- or 6-nicotinyl - linker - carrier protein are 5-(1-methyl-2-pyrrolidinyl)-2- or 3-pyridinyl - linker - carrier protein and 5-(N-methyl-2-pyrrolidinyl)-2- or 3-pyridinyl - linker - carrier protein.

The carrier protein of the 5- or 6-nicotinyl - linker - carrier protein of the invention may be selected from pharmaceutically acceptable proteins which coupled to a hapten are suitable for eliciting antibodies in humans, and is for example selected from the group consisting of keyhole limpet hemocyanin (KLH), tetanus toxoid, diphtheria toxoid, non-toxic mutant diphtheria toxoid $CRM_{197}$, outer membrane protein complex (OMPC) from Neisseria meningitidis, the B subunit of heat-labile Escherichia coli, and recombinant exoprotein A from Pseudomonas aeruginosa (rEPA).

The present invention is further directed to a 5- or 6 - nicotinyl -linker - carrier protein having the formula




wherein

X is -NH-CO- or -NH- or -C≡C- or -C=C- or $-CH_2-$ ;

Y is $-(CH_2)_k-$ or $-(CH_2)_m-C_6H_{10}-(CH_2)_n-$ or $-(CH_2)_m-C_6H_4-(CH_2)_n-$

wherein k = 0-20, m = 0 - 6, and n = 0 – 6, when

Z is -NH-,

with the provisio that X is not –NH-CO- when Y is $(CH_2)_5$ and Z is -NH-,

and

X is -NH-CO- or -C≡C- or -C=C- or $-CH_2-$,

Y is $-(CH_2)_m-C_6H_{10}-(CH_2)_n-$ or $-(CH_2)_m-C_6H_4-(CH_2)_n-$

wherein m = 0 - 6, and n = 0 – 6, when

Z is -CO-,

and

X is -C≡C- or -C=C- ,

Z is -CO-, when

Y is $-(CH_2)_k-$

wherein k = 0 – 20.

Also in this aspect of the invention the carrier protein may be selected from the group consisting of keyhole limpet hemocyanin (KLH), tetanus toxoid, diphtheria toxoid, non-toxic mutant diphtheria toxoid $CRM_{197}$, outer membrane protein complex (OMPC) from Neisseria meningitidis, the B subunit of heat-labile Escherichia coli, and recombinant exoprotein A from Pseudomonas aeruginosa (rEPA).

Further, the invention is directed to the new 5- or 6 - nicotinyl -linker - carrier proteins according to the invention for use as a medicament, such as the immunizing component in a vaccine.

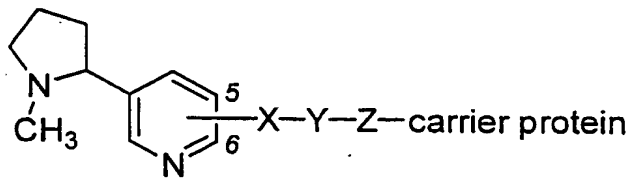
One compound excluded from the compounds of the above formula of the invention has been previously disclosed by Castro A. and Monji N. (ibid.), namely the 6-nicotinyl- linker - carrier protein wherein the carrier protein is bovine serum albumin and the linker is - $NH-CO-(CH_2)_5-NH-$. However, this compound has not been suggested for medical use.

The invention is additionally directed to a pharmaceutical composition comprising a 5- or 6 - nicotinyl -linker - carrier protein according to the invention and a pharmaceutically acceptable vehicle.

The vehicle in the pharmaceutical composition needed for administration of the immunogenic compound of the invention may be selected from known pharmaceutically acceptable vehicles, such as physiological saline solution or other suitable vehicle e.g. disclosed in the European or US Pharmacopoeia.

The pharmaceutical composition according to the invention may further comprise an adjuvant, which naturally must be pharmaceutically acceptable for human use, such as aluminum phosphate and aluminum hydroxide.

The invention is furthermore directed to a method of prophylactic and/or therapeutic immunological treatment of nicotine dependence from tobacco products to achieve harm reduction in an individual comprising administration of a 5- or 6 - nicotinyl -linker - carrier protein having the formula




wherein

X is -NH-CO- or -NH- or -C≡C- or -C=C- or -$CH_2$- ;

Y is -$(CH_2)_k$- or -$(CH_2)_m$-$C_6H_{10}$-$(CH_2)_n$- or -$(CH_2)_m$-$C_6H_4$-$(CH_2)_n$-

wherein k = 0-20, m = 0 - 6, and n = 0 – 6, when

Z is -NH-,

with the provisio that X is not –NH-CO- when Y is $(CH_2)_5$ and Z is -NH-,

and

X is -NH-CO- or -C≡C- or -C=C- or -$CH_2$-,

Y is -$(CH_2)_m$-$C_6H_{10}$-$(CH_2)_n$- or -$(CH_2)_m$-$C_6H_4$-$(CH_2)_n$-

wherein m = 0 - 6, and n = 0 – 6, when

Z is -CO-,

and

X is -C≡C- or -C=C- ,

Z is -CO-, when

Y is -$(CH_2)_k$-

wherein k = 0 – 20,

to said individual in antibody-eliciting amounts for eliciting antibodies binding to nicotine molecules.

For a specific individual the antibody-eliciting amount will be found empirically by subjective experience of the stimulating effect in error and trial tests, or will be suggested by the manufacturer or physician.

In a preferred embodiment of this aspect of the invention the administration is repeated at intervals to enhance the titre of antibodies binding to nicotine molecules in said individual.

The individual is preferably a human even though the invention would function if another mammal should be treated. This could of course be the case if laboratory animals were used for testing purposes.

The present invention will now be further illustrated by reference to the following description of drawings, synthesis of compounds and immunogens and experiments which describe specific embodiments of the invention. However, these are not to be considered as limitations to the scope of the invention defined in the claims.

**Description of the drawings**

Figure1. Elisa measurements on serum from immunized rats collected 7 or 9 days after the first bolus immunization with GK5-KLH.

Figure 2. Elisa measurements on serum from immunized rats collected 1-4 days after booster immunization with GK5-KLH.

Figure 3. Elisa titre measurements of serum from immunized rats. The titre of specific antibodies from GK60-KLH immunized rats is 1:4000. Immunization using other immunogens gave similar results.

Figure 4. Competitive Elisa. Immunogen GK-84-KLH immunized using protocol 2 gave rise to antibodies that were about 100 times more specific to nicotine and nornicotine than to the other competitors used.

Figure 5. Electrophysiological single-cell recordings from VTA DA neurones in control rats. The DA cells responded with an increase in firing rate and burst firing after nicotine administration.

Figure 6. Electrophysiological single-cell recordings from VTA DA neurones in immunized rats. The DA cells appear less sensitive to the activating effects of nicotine after immunization.

Figure 7a. Active immunization using immunogen GK84-KLH essentially completely suppresses nicotine-induced dopamine release in nucleus accumbens shell compared to controls.

Figure 7b. Active immunization using immunogen GK80-KLH results in a marked increase on the dopamine overflow in nucleus accumbens shell after nicotine administration compared to controls.

Figure 7c. The effect of the immunization with immunogen YH6-KLH on nicotine-induced dopamine output is stable over a three week period. * p=0.05; ** p=0.01; *** p=0.001.

**Description of synthesis of compounds and immunogens**

The nicotine immunogens were prepared in three steps according to Scheme 1:

I. preparation of 6-aminonicotine (1) from (*S*)-nicotine,

II. preparation of various nicotine-linker molecules,

III. preparation of various nicotine immunogens (nicotine-linker-carrier protein).

Experimental details of the three steps follow below:

## I. Preparation of 6-aminonicotine (1).

6-Aminonicotine (**1**) was synthesized from (*S*)-nicotine according to the procedure reported by Tschitschibabin and Kirssanow *Chem. Ber.* **1924**, *57B*, 1163-1168.

## II. Preparation of various nicotine-linker molecules (Schemes 2-8)

***General procedure for coupling of 6- or 5-aminonicotine with amine derivatives as linkers (Schemes 2-3):*** *N*-Ethyldiisopropylamine (DIEA, 3.0 equiv) was added to a mixture of 6-aminonicotine (1.0 equiv), an amino protected amino acid [7-(*tert*-butyloxycarbonylamino)-heptanoic acid or *trans*-4-(benzyl-oxycarbonylaminomethyl)cyclohexanecarboxylic acid[1]] (1.0 equiv) and tripyrrolidinobromo-phosphonium hexafluorophosphate (PyBrOP, 1.0 equiv) in $CH_2Cl_2$ (1-2 mL/mmol). The reaction mixture was stirred at ambient temperature for 20-24 h. The mixture was concentrated and the residue was chromatographed to give the 6-acylated 6-aminonicotine derivatives **2** and **3** in 32-38% yield.

[1]Okano, A.; Inaoka, M.; Funabashi, S.; Iwamoto, M.; Isoda, S.; Moroi, R.; Abiko, Y.; Hivata, M. *J. Med. Chem.* **1972**, *15*, 247-255.

**7-(*tert*-Butyloxycarbonylamino)-*N*-[5-(1-methyl-2-pyrrolidinyl)-2-pyridinyl]heptan-amide (2).** The residue was chromatographed [silica gel, $CHCl_3$/MeOH (30:1)] to give **2** (32%). An analytical sample was obtained by crystallization from *iso*-hexane/EtOH: mp 92-93 °C; $^1$H-NMR ($CDCl_3$, 270 MHz) $\delta$9.34, (br s, 1H), 8.16-8.11 (m, 2H), 7.63, (dd, $J$=8.5, 2.5 Hz, 1H), 4.68 (br s, 1H), 3.20-3.10 (m, 1H), 3.08-2.90 (m, 3H), 2.32 (t, $J$=7.5 Hz, 2H), 2.30-2.15 (m, 1H), 2.15-2.00 (m, 1H), 2.07 (s, 3H), 1.96-1.55 (m, 5H), 1.45-1.20 (m, 6H), 1.36 (s, 9H); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$171.9, 155.8, 150.9, 146.5, 137.5, 134.3, 114.2, 78.7, 68.4; 56.7, 40.3, 40.1, 37.2, 35.0, 28.7, 28.3, 26.3, 25.1, 22.3.

***trans*-4-(Benzyloxycarbonylaminomethyl)-*N*-[5-(1-methyl-2-pyrrolidinyl)-2-pyridinyl]cyclohexanecarboxamide (3).** The residue was chromatographed [silica gel, EtOAc/MeOH (20:1)] to afford **3** (38%). An analytical sample was obtained by crystallization from *iso*-hexane/EtOH: mp 173-174 °C; $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$8.76 (s, 1H), 8.22-8.18 (m, 2H), 7.70 (dd, $J$=2, 8.5 Hz, 1H), 7.40-7.30 (m, 5H), 5.10 (s, 2H), 4.94-4.90 (m, 1H),

3.27-3.18 (m, 1H), 3.09-2.99 (m, 3H), 2.35-2.10 (m, 3H), 2.15 (s, 3H), 2.06-1.4 (m, 10H), 1.07-0.88 (m, 2H); $^{13}$C NMR ($CD_3OD$, 100 MHz) $\delta$174.4, 156.5, 150.8, 146.7, 137.6, 136.5, 134.6, 128.4 (2C's), 128.0 (3C's), 114.1, 68.5, 66.6, 56.9, 47.0, 46.1, 40.3, 37.5, 35.1, 29.6 (2C's), 28.8 (2C's), 22.4;

**7-Amino-*N*-[5-(1-methyl-2-pyrrolidinyl)-2-pyridinyl]heptanamide (4:YH15).** TFA (5 mL) was added to a solution of **2** (0.10 g, 0.25 mmol) in $CH_2Cl_2$ (20 mL) and the mixture was stirred at ambient temperature for 2h. The solution was neutralized with saturated aqueous $K_2CO_3$ and extracted with $CH_2Cl_2$ (3 x 15 mL). The combined organic phases was washed with water, dried ($K_2CO_3$), filtered and concentrated. The crude product was purified by preparative TLC [silica gel, $CHCl_3$/MeOH/$NH_4OH$ (50:50:1)] to afford 0.06 g (74%) of **4** as an oil; $^1$H NMR ($CD_3OD$, 400 MHz) $\delta$8.21 (d, $J$=2 Hz, 1H), 8.07, (d, $J$=8.5 Hz, 1H), 7.74 (dd, $J$=8.5, 2.5 Hz, 1H), 3.23-3.17 (m, 1H), 3.13-3.08 (m, 1H), 2.65-2.60 (m, 2H), 2.42 (t, $J$=7.5 Hz, 2H), 2.36-2.28 (m, 1H), 2.23-2.16 (m, 1H), 2.14 (s, 3H), 2.00-1.66 (m, 5H), 1.52-1.33 (m, 6H); $^{13}$H NMR ($CD_3OD$, 100 MHz) $\delta$174.8, 152.5, 148.5, 138.5, 134.7, 115.5, 69.8, 57.8, 42.4, 40.5, 37.8, 35.3, 33.4, 30.1, 27.7, 26.6, 23.1;

***trans*-4-(Aminomethyl)-*N*-[5-(1-methyl-2-pyrrolidinyl)-2-pyridinyl]cyclohexanecarboxamide (5:YH6).** A mixture of **3** (0.14 g, 0.3 mmol), $Me_3SiCl$ (0.24 g, 2.25 mmol) and NaI (4.5 mg, 2.7 mmol) in dry acetonitrile (10 mL) was stirred at ambient temperature for 3h under nitrogen. The volatiles were evaporated and MeOH (5 mL) and a saturated solution of HCl in ether (1 mL) were added. The mixture was stirred for 15 min and then concentrated. The residue was diluted with water (5 mL) and extracted with EtOAc (3 x 7 mL). The combined organic phases was dried ($K_2CO_3$), filtered and concentrated. The crude product was purified by preparative TLC [silica gel, $CHCl_3$/MeOH/$NH_4OH$ (50:50:1)] to afford 0.03 g (28%) of **5**; $^1$H-NMR ($CDCl_3$, 270 MHz) $\delta$ 8.83 (br s, 1H), 8.17-8.09 (m, 2H), 7.64 (dd, $J$=2, 8.5 Hz, 1H), 3.20-3.10 (m, 1H), 2.96 (t, $J$=8 Hz, 1H), 2.49 (br s, 2H), 2.08 (s, 3H), 2.28-1.18 (m, 15H), 0.94-0.84 (m, 2H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$174.6, 150.9, 146.6, 137.6, 134.5, 114.1, 68.4, 56.8, 48.3, 46.3, 40.2, 35.1, 29.7 (2C's), 29.6, 28.9 (2C's), 22.4;

**6-[5-(1-methyl-2-pyrrolidinyl)-2-pyridinyl]hexanenitrile (6).** A stirred mixture of 6-aminonicotine (0.62 g, 3.5 mmol) and NaH (80% in mineral oil, 0.16 g, 5.25 mmol) in dry toluene (20 mL) was refluxed for 2h and then cooled to room temperature. 6-Bromohexane-nitrile (0.65 g, 3.7 mmol) was added and the reaction was left at ambient temperature over night. The solvent was evaporated and the residue was chromatographed [silica gel, $CHCl_3$/MeOH/$NH_4OH$ (18:3:0.1)] to afford 0.40 g (41%) of **6**; IR (film): 2270 cm$^{-1}$ (CN); $^1$H-

NMR ($CDCl_3$, 270 MHz) $\delta$7.95 (d, $J$=2.5 Hz, 1H), 7.47 (dd, $J$=2.5, 8.5 Hz, 1H), 6.39 (d, $J$=8.5 Hz, 1H), 4.49 (m, 1H), 3.36-3.16 (m, 3H), 2.90 (t, $J$=9 Hz, 1H), 2.36 (t, $J$=7 Hz, 2H), 2.29-2.19 (m, 1H), 2.19-2.06 (m, 1H), 2.14 (s, 3H), 2.00-1.50 (m, 9H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$158.2, 147.4, 136.5, 126.4, 119.4, 106.9, 68.5, 56.7, 41.7, 40.0, 34.3, 28.7, 26.0, 25.0, 22.1, 16.7;

**6-[5-(1-methyl-2-pyrrolidinyl)-2-pyridinyl]hexanamine (7:YH7)** Compound **6** (0.23 g, 0.83 mmol) was added to a suspension of $LiAlH_4$ (0.07 g, 2.5 mmol) in dry ether (8 mL) under nitrogen. The mixture was stirred at room temperature for 5h, then heated under reflux for 20h. Saturated aqueous $Na_2SO_4$ (2 mL) was added and the solid aluminium complex was filtered and washed with ether. The organic layer was dried ($K_2CO_3$), filtered and concentrated. The crude residue was purified by preparative TLC [silica gel, $CHCl_3$/MeOH/$NH_4OH$ (35:35:2)] to afford 0.13 g (55%) of **7**; $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 7.83 (d, $J$=2 Hz, 1H), 7.47 (dd, $J$=2, 9 Hz, 1H), 6.55, (d, $J$=9 Hz, 1H), 3.27 (t, $J$=7 Hz, 2H), 3.25-3.15 (m, 1H), 3.00-2.90 (m, 1H), 2.70 (br s, 2H), 2.35-2.26 (m, 1H) 2.21-2.09 (m, 1H), 2.16 (s, 3H), 2.10-1.74 (m, 3H), 1.69-1.35 (m, 8H); $^{13}$C NMR ($CD_3OD$, 100 MHz) $\delta$160.3, 147.8, 138.0, 125.5, 110.1, 70.2, 57.8, 42.8, 42.3, 40.5, 34.6, 33.0, 30.5, 28.2, 27.8, 22.9;

***trans*-Benzyl 4-[5-(1-methyl-2-pyrrolidinyl)-2-pyridinylcarbamoyl]cyclohexane-carboxylate (8).** DIEA (0.51 ml, 3 mmol) was added to a mixture of 6-aminonicotine (0.27 g, 1.5 mmol), 4-(benzyloxycarbonyl)cyclohexancarboxylic acid[2] (0.43 g, 1.6 mmol), and PyBrOP (0.77 g, 1.6 mmol) in $CHCl_3$ (20 ml). The reaction mixture was stirred at ambient temperature for 5 min and then heated under reflux for 24 h. Chloroform was evaporated *in vacuo* and the residue was extracted with ether. The combined ether extracts was washed with water followed by brine and then dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was chromatographed [silica gel, $CH_2Cl_2$/MeOH (95:5)], to give **8** (0.44 g, 70%). An analytical sample was obtained by crystallization from *iso*-hexane; mp 116-118 °C; MS (EI, 20 *e*V) *m/e* 422 ($M^+$ + 1), 421 ($M^+$), 420 ($M^+$ - H); $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$8.61 (br s, 1H), 8.20-8.15 (m, 2H), 7.70 (dd, $J$=8.5, 2.5 Hz, 1H), 7.34 (m, 5H), 5.12 (s, 2H), 3.22 (m, 1H), 3.03 (app t, 1H), 2.45 - 1.40 (m, 15H), 2.16 (s, 3H); $^{13}$C NMR ($CDCl_3$, 68 MHz): $\delta$175.1, 174.1, 150.8, 146.8, 137.7, 136.0, 134.8, 128.5 (2C's), 128.1, 128.0 (2C's), 114.2, 68.5, 66.1, 56.9, 45.4, 42.3, 40.3, 35.1, 28.4 (2C's), 27.9 (2C's), 22.4;

[2]Wellmar, U. et al. *Nucleosides Nucleotides*, **1996**, *15*, 1059-1076.

***trans*-4-[5-(1-Methyl-2-pyrrolidinyl)-2-pyridinylcarbamoyl]cyclohexanecarboxylic acid (9:GK5)**: Palladium on carbon (10 %, 0.04 g) was added to a mixture of **8** (0.15 g, 0.35

mmol) and cyclohexene (0.15 mL) in EtOH (7 mL). The stirred reaction mixture was heated under reflux for 15 min, filtered and concentrated. The residue was purified by preparative TLC (silica gel, MeOH), dissolved in $CH_2Cl_2$/EtOH (95:5), filtered and concentrated to yield **9** (0.09 g, 75%); MS (EI, 20 *e*V) *m/e* 332 ($M^+$ + 1), 331 ($M^+$), 330 ($M^+$ - H); $^1$H NMR ($CDCl_3$, 270 MHz): $\delta$10.8 (br s, 1H), 8.34 (d, *J*=8.5 Hz, 1H), 8.06 (s, 1H), 7.79 (d, *J*=8.5 Hz, 1H), 3.23 (app t, 1H), 3.05 (app t, 1H), 2.60 - 1.50 (m, 15H), 2.12 (s, 3H); $^{13}$C NMR ($CDCl_3$+$CD_3OD$, 68 MHz): $\delta$179.6, 175.2, 151.0, 146.2, 138.1, 132.2, 114.4, 68.4, 56.3, 44.9, 42.7, 39.5, 33.7, 28.2, 28.0, 21.8;

**7-(*tert*-Butyloxycarbonylamino)-*N*-[5-(1-methyl-2-pyrrolidinyl)-3-pyridinyl]heptanamide (SG17).** PyBrOP (0.9 g, 1.9 mmol) and DIEA (0.6 ml) was added to a stirred solution of 5-aminonicotine[3] (0.3 g, 1.7 mmol) and 7-(*tert*-butyloxycarbonylamino)heptanoic acid (0.5 g, 2.0 mmol) in dry $CH_2Cl_2$ (5 ml) at 0 °C. The mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. The solvent was evaporated and EtOAc was added to the residue. The mixture was washed with aqueous saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was chromatographed [$SiO_2$, $CHCl_3$/MeOH (4:1)], to afford 0.59 g (87%) of **SG17** as a light yellow oil; $^1$H-NMR (270 MHz, $CDCl_3$) $\delta$8.83 (br s, 1H), 8.59 (d, *J*=2 Hz, 1H), 8.27 (br s, 1H), 8.15 (br s, 1H), 4.75 (br s, 1H), 3.21 (ddd, *J*=9.5, 9.5, 2 Hz, 1H), 3.15-3.04 (m, 3H), 2.39-2.13 (m, 7H), 1.95-1.65 (m, 5H), 1.50-1.31 (m, 15H); $^{13}$C-NMR (68 MHz, $CDCl_3$) $\delta$172.5, 156.4, 144.4, 140.3, 139.2, 135.7, 126.4, 79.4, 68.9, 57.1, 40.5, 37.2, 35.2, 30.1, 28.6 (3 Cs), 26.3, 25.5, 22.8;

[3]L. Röndahl. *Acta Pharm. Suec.* **1977**, *14*, 113-118.

**7-Amino-*N*-[5-(1-methyl-2-pyrrolidinyl)-3-pyridinyl]heptanamide (SG18).** TFA (2.0 ml, 26.0 mmol) was added to a stirred solution of **SG17** (0.48 g, 1.2 mmol) in dry $CH_2Cl_2$ (35 ml) at 0 °C. The stirred solution was allowed to reach room temperature and after 6 h the solution was brought to pH~8 by addition of aqueous saturated $K_2CO_3$. The mixture was evaporated to complete dryness under reduced pressure. The residue was chromatographed [$SiO_2$, $CHCl_3$/MeOH/$NH_4OH$ (4:1:0.5] to afford 0.25 g (69%) of **SG18** as a yellow oil; $^1$H-NMR (270 MHz, $CDCl_3$) $\delta$9.30 (br s, 1H), 8.59 (br s, 1H), 8.22-8.13 (m, 2H), 3.88 (br s, 2H), 3.16-3.12 (m, 1H), 3.08-3.02 (m, 1H), 2.71 (br s, 2H), 2.39-2.12 (m, 7H), 1.87-1.64 (m, 5H), 1.47-1.31 (m, 6H); $^{13}$C-NMR (68 MHz, $CDCl_3$) $\delta$172.7, 144.1, 140.0, 139.7, 135.9, 126.6, 68.8, 57.1, 41.5, 40.6, 37.1, 35.3, 31.9, 28.8, 26.4, 25.4, 22.8;

***Preparation of some linkers (Scheme 4)***

***trans*-(4-Prop-2-ynylcyclohexyl)methanol (GK91).** Lithium acetylide ethylenediamine complex (7.4 g, 72.5 mmol) was suspended in 40 ml of dry DMSO and a solution of *trans*-4-

(hydroxymethyl)cyclohexylmethyl toluene-4-sulfonate (8.0 g, 26.8 mmol) in DMSO (30 ml) was added at 5 °C. The mixture was stirred for 2 h at ambient temperature, quenched with cold $H_2O$ (200 ml) and filtered through a pad of celite. The resulting solution was extracted with ether (4 x 50 ml). The combined organic layers was washed with water and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* yielding 3.5 g (86 %) of **GK91** which was used in the next step without further purification; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$3.44 (d, $J$=6 Hz, 2H), 2.10 (dd, $J$=6.5, 2.5 Hz, 2H) 1.97 (t, $J$=2.5 Hz, 1H), 1.86 (m, 4H), 1.72 (br s, 1H), 1.43 (m, 2H), 1.01 (m, 4H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$ 83.3, 69.0, 68.4, 40.1, 37.1, 31.8, 29.1, 26.0. A portion of this material was converted to the 3,5-dinitrobenzoate. mp 106-108 °C (from *iso*-hexane); $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$9.23 (t, $J$=2 Hz, 1H), 9.15 (d, $J$=2 Hz, 2H), 4.29 (d, $J$=6.5 Hz, 2H), 2.15 (dd, $J$= 6.5, 2.5 Hz, 2H), 1.98 (t, $J$=2.5 Hz, 1H), 1.92 (m, 4H), 1.85 (m, 1H), 1.52 (m, 1H), 1.14 (m, 4H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$162.5, 148.7, 134.1, 129.3, 122.3, 82.9, 71.7, 69.3, 36.9, 36.8, 31.4, 29.2, 25.9;

***trans*-4-Prop-2-ynylcyclohexanecarboxylic acid. (GK68).** A solution of chromium trioxide (1.18 g, 11.8 mmol) in 2M $H_2SO_4$ (10 ml, 20 mmol) was maintained at 0 °C while 0.9 g (~5.9 mmol) of **GK91** in acetone (20 ml) was added. The reaction mixture was stirred for 1 h at 0 °C and for 2 h at room temperature. The excess of oxidizing agent was destroyed with solid $Na_2SO_3$ (0.8 g, 6.3 mmol). Ether (~100 ml) was added and the mixture was extracted with brine (3 × 50 ml). The organic phase was dried ($Na_2SO_4$), filtered and concentrated. The residue was crystallized from *iso*-hexane to give 0.74 g (75%) of **GK68** as colorless crystals. mp 84–86 °C (*iso*-hexane); $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$10.4 (br s, 1H), 2.27 (app tt, 1H), 2.12 (dd, $J$=6.5, 2.5 Hz, 2H), 1.98 (t, $J$=2.5 Hz, 1H), 2.15-1.87 (m, 4H), 1.6-1.35 (m, 3H), 1.09 (m, 2H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$182.4, 82.8, 69.4, 42.8, 36.2, 31.3, 28.4, 25.8;

***trans*-4-Prop-2-ynylcyclohexanecarboxylic acid methyl ester. (GK70). GK68** (0.7 g, 4.2 mmol) was dissolved in a mixture of MeOH (5 mL) and trimethyl orthoformate (1 mL). Dowex 50W X2 (0.2 g) was added and the reaction mixture was stirred overnight at ambient temperature. The resin was filtered off and the solvents were evaporated *in vacuo*. The residue was purified by bulb to bulb distillation (175 °C, 13 mm) to give 0.71 g (93 %) of **GK70** as an oil; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$3.66 (s, 3H), 2.24 (app tt, 1H), 2.11 (dd, $J$=6.5, 2.5 Hz, 2H), 2.07-1.85 (m, 4H), 1.97 (t, $J$=2.5 Hz, 1H), 1.6-1.35 (m, 3H), 1.06 (m, 2H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$176.3, 82.8, 69.3, 51.5, 43.0, 36.2, 31.4, 28.7, 25.8;

***trans*-(4-Prop-2-ynylcyclohexyl)methyl toluene-4-sulfonate(GK87).** A solution of **GK91** (3.04 g, 20 mmol) in pyridine (30 ml) was cooled to 0 °C and *p*-toluenesulfonyl chloride (4 g,

21 mmol) was added. The reaction mixture was stirred for 2 h at 0 °C and overnight at ambient temperature. The pyridine was evaporated *in vacuo* and the residue was dissolved in ether, washed with 1M HCl, $H_2O$, $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and evaporated. The solid residue was recrystallized from *iso*-hexane to give 5.57 g (91%) of **GK87**: mp 59-60 °C; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$7.78 (m, 2H), 7.34 (m, 2H), 3.82 (d, $J$=6.5 Hz, 2H), 2.45 (s, 3H), 2.07 (dd, $J$=6.5, 2.5 Hz, 2H), 1.95 (t, $J$=2.5 Hz, 1H), 1.80 (m, 4H), 1.60 (m, 1H), 1.41 (m, 1H), 0.97 (m, 4H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$144.6, 133.1, 129.8, 127.8, 82.9, 75,1, 69.2, 37.0, 36.7, 31.3, 28.7, 25.8, 21.6;

***trans*-(4-Prop-2-ynylcyclohexyl)methylamine (GK88).** A solution of **GK87** (1.53 g, 5 mmol) in MeOH (50 ml) was saturated with $NH_3$ at -15 °C and then heated in a sealed vessel at 100 °C for 3 h. The MeOH was evaporated *in vacuo*. The residue was dissolved in 1M HCl (50 ml) and extracted with $CH_2Cl_2$ (3 × 20 ml) to remove *bis*(*trans*-(4-prop-2-ynyl-cyclohexylmethyl)amine hydrochloride. The water phase was alkalinized with solid NaOH to pH12 and extracted with ether (3 × 20 ml). The combined organic extracts were dried over KOH, filtered and concentrated *in vacuo* to give 0.4 g (53%) of **GK88**; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$2.51 (br d, 2H), 2.07 (m, 2H), 1.94 (m, 1H), 1.83 (m, 4H), 1.42 (m, 1H), 1.3–0.8 (m, 7H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$83.3, 68.9, 48.6, 41.0, 37.2, 32.0, 30.3, 26.0;

**6-Bromohexylammonium bromide[4] (SG20).** A mixture of 6-amino-1-hexanol (4.3 g, 37.0 mmol) in HBr 48% (105 ml) was stirred at 95 °C for 11h. The solution was concentrated *in vacuo* to afford 8.5 g (89%) of **SG20** as a brownish hygroscopic powder (mp of crude **SG20**: 128-132 °C); $^1$H-NMR (270 MHz, $CD_3COCD_3$) $\delta$8.30 (br s, 2H), 3.53 (t, $J$=7 Hz, 1H), 3.52 (t, $J$=7 Hz, 1H), 3.13-3.06 (m, 2H), 1.91-1.87 (m, 4H), 1.54-1.46 (m, 4H); $^{13}$C-NMR (68 MHz, $CD_3COCD_3$) $\delta$40.5, 34.8, 33.4, 28.3, 27.7, 26.4;

[4]Dumont, J.L.; Chodkiewicz, W.; Cadiot, P. *Bull. Soc. Chim. Fr.* **1967**, *2*, 558-596.

**7-Octynamine[5] (SG26).** A solution of **SG20** (3.0 g, 11.5 mmol) in dry DMSO (2 ml) was added dropwise over a period of 45 min to a stirred slurry of lithium acetylide, ethylendiamine complex (7.0 g, 76.0 mmol) in dry DMSO (36 ml) at 8 °C. After 3 h at 8 °C the slurry was slowly poured into a cooled solution of brine (*ca* 300 ml). The solution was extracted with hexane (3x150 ml) and the combined organic phases was dried over $Na_2SO_4$, filtered and concentrated *in vacuo* at room temperature to afford 1.0g (69%) of **SG26** as a light yellow oil; IR (film) $\nu_{max}$ 2114, 3296 cm$^{-1}$; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$2.63 (t; $J$=7 Hz, 2H), 2.14 (dt; $J$=7, 2.5 Hz, 2H), 1.88 (t; $J$=2.5 Hz, 1H), 1.39-1.31 (m, 10H); $^{13}$C NMR (68 MHz, $CDCl_3$) $\delta$ 84.6, 68.3, 42.2, 33.7, 28.6, 28.5, 26.4, 18.4;

[5]Novis Smith, W. and Beumel, O.F. *Synthesis*, **1974**, 441-442.

***General procedure for coupling of 6-bromonicotine and 5-bromonicotine with terminal alkynes. (Scheme 5)*** A mixture of 6-bromonicotine[6] (or 5-bromonicotine[7]) (0.24 g, 1 mmol), bis(triphenyl-phosphine)palladium dichloride (0.014 g, 0.02mmol) and CuI (0.004 g, 0.02mmol) in 5 ml of $Et_3N$ was deoxygenated with $N_2$. The acetylenic compound (1.1 mmol) was added and the reaction mixture was heated at 120 °C for 40 min (4 h for 5-bromonicotine) in a sealed vessel. The $Et_3N$ was evaporated *in vacuo* and the residue was dissolved in EtOAc, washed with saturated aqueous $NaHCO_3$ and extracted with 20 ml of 2*N* HCl. The acidic aqueous phase was extracted with EtOAc (4×20 ml). The aqueous layer was saturated with solid $NaHCO_3$ and extracted with EtOAc (3×30 ml). The organic phase was washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by column chromatography.

[6]M. Dukat, W. Fiedler, D. Dumas, I. Damaj, B.R. Martin, J.A. Rosecrans, J.R. James, R.A. Glennon. *Eur. J. Med. Chem.* **1996**, *31*, 875-888. [7] L.S. Bleicher, N.D.P. Cosford, A. Herbaut, J.S. McCallum and I.A. McDonald. *J.Org.Chem.* **1998**, *63*, 1109-1118.

**Methyl 4-[(5-(1-methyl-2-pyrrolidinyl)-2-pyridinyl)ethynyl]benzoate (GK47).** Methyl 4-ethynylbenzoate was prepared according to ref 8. Purification: column chromatography [silica, acetone/*iso*-hexane (1:2)]; yield 78%; mp 102-104 °C (*iso*-hexane); $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$8.56 (br s, 1H), 8.03 (dd, *J*=8.5, 1.5 Hz, 2H), 7.72 (app dt, 1H), 7.63 (dd, *J*=8.5, 1.5 Hz, 2H), 7.52 (d, *J*=8 Hz, 1H), 3.92 (s, 3H), 3.25 (m, 1H), 3.13 (t, *J*=8 Hz, 1H), 2.4-2.1 (m, 5H), 2.1-1.6 (m, 3H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$166.3, 149.9, 141.6, 138.9, 135.1, 131.8, 130.0, 129.4, 127.2, 127.0, 91.3, 87.7, 68.6, 56.9, 52.2, 40.3, 35.3, 22.6;

[8]S.J. Havens and P.M. Hergenrother. *J. Org. Chem.* **1985**, 50, 1763-1765.

**Methyl 7-(5-(1-Methyl-2-pyrrolidinyl)-2-pyridinyl)hept-6-ynoate. (GK79).** Methyl 6-heptynoate was prepared according to ref 9. Purification: column chromatography [silica, acetone/*iso*-hexane (1:2)]; yield 73%; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$8.45 (d, *J*=2 Hz, 1H), 7.63 (dd, *J*=8, 2 Hz, 1H), 7.33 (d, *J*=8 Hz, 1H), 3.67 (s, 3H), 3.23 (m, 1H), 3.08 (app t, 1H), 2.47 (t, *J*=7 Hz, 2H), 2.37 (t, *J*=7 Hz, 2H), 2.31 (m, 1H), 2.18 (m, 1H), 2.16 (s, 3H), 2.05–1.6 (m, 7H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$173.7, 149.5, 142.5, 137.7, 134.9, 126.6, 89.6, 80.7, 68.6, 56.9, 51.4, 40.3, 35.1, 33.5, 27.7, 24.1, 22.5, 19.0;

[9]E.C. Taylor et al. *J. Org. Chem.* **1991**, 56, 1807-1812.

**Methyl 7-(5-(1-Methyl-2-pyrrolidinyl)-3-pyridinyl)hept-6-ynoate. (GK77). ).** Methyl 6-heptynoate was prepared according to ref 9. Purification: column chromatography [silica,

acetone/*iso*-hexane (1:2)]; yield 60%; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$8.51 (br s, 1H), 8.42 (br s, 1H), 7.71 (br s, 1H), 3.68 (s, 3H), 3.24 (m, 1H), 3.08 (app t, 1H), 2.46 (t, *J*=7 Hz, 2H), 2.38 (t, *J*=7 Hz, 2H), 2.31 (m, 1H), 2.20 (m, 1H), 2.17 (s, 3H), 2.1–1.55 (m, 7H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$173.8, 151.0, 147.8, 138.2, 137.3, 120.8, 92.9, 77.9, 68.5, 56.9, 51.5, 40.3, 35.1, 33.5, 27.9, 24.1, 22.6, 19.1;

***trans*-4-[3-(5-(1-Methyl-2-pyrrolidinyl)-2-pyridinyl)prop-2-ynyl]cyclohexanecarboxylic acid methyl ester (GK78).** Purification: column chromatography [silica, acetone/*iso*-hexane (1:2)]; yield 87%; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$8.46 (d, *J*=2 Hz, 1H), 7.63 (dd, *J*=8, 2 Hz, 1H), 7.34 (d, *J*=8 Hz, 1H), 3.66 (s, 3H), 3.24 (m, 1H), 3.08 (app t, 1H), 2.4–2.1 (m, 8H), 2.1–1.3 (m, 10H), 1.15 (m, 2H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$176.3, 149.5, 142.6, 137.7, 134.9, 126.7, 88.7, 81.5, 68.6, 56.9, 51.5, 43.0, 40.3, 36.4, 35.1, 31.6, 28.7, 26.8, 22.6;

***trans*-4-[3-(5-(1-Methyl-2-pyrrolidinyl)-3-pyridinyl)prop-2-ynyl]cyclohexanecarboxylic acid methyl ester (GK75).** Purification: column chromatography [silica, acetone/*iso*-hexane (1:2)]; yield 58%; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$8.50 (d, *J*=2 Hz, 1H), 8.41 (d, *J*=2 Hz, 1H), 7.71 (d, *J*=2 Hz, 1H), 3.67 (s, 3H), 3.25 (m, 1H), 3.08 (app t, 1H), 2.4–2.1 (m, 8H), 2.1–1.3 (m, 10H), 1.14 (m, 2H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$176.3, 151.1, 147.8, 138.1, 137.2, 120.8, 91.9, 78.6, 68.5, 56.9, 51.5, 43.0, 40.3, 36.5, 35.1, 31.6, 28.7, 26.9, 22.6;

**2-(3-Hydroxy-3-methylbut-1-ynyl)-5-(1-methyl-2-pyrrolidinyl)pyridine (GK55).** Purification: column chromatography [silica, acetone/*iso*-hexane (1:1)]; yield 90%; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$8.47 (d, *J*=2 Hz, 1H); 7.67 (dd, *J*=8, 2 Hz, 1H), 7.37 (d, *J*=8 Hz, 1H), 3.50 (br s, 1H) 3.23 (m, 1H), 3.08 (app t, 1H), 2.30 (m, 1H), 2.19 (m, 1H), 2.15 (s, 3H), 2.05–1.55 (m, 3H), 1.64 (s, 6H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$149.4, 141.7, 138.3, 135.1, 127.0, 94.0, 81.2, 68.6, 65.0, 56.9, 40.3, 35.1, 31.2, 22.6;

***trans*-4-[3-(5-(1-Methyl-2-pyrrolidinyl)-3-pyridinyl)-prop-2-ynyl]cyclohexylmethyl-amine (GK89).** Purification: column chromatography [silica, $CHCl_3$ : MeOH saturated with $NH_3$ (15:1)]; yield 90%; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$8.50 (d, *J*=2 Hz, 1H), 8.40 (d, *J*=2 Hz, 1H), 7.70 (t, *J*=2 Hz, 1H), 3.24 (m, 1H), 3.07 (app t, 1H), 2.55 (d, *J*=6.5 Hz, 2H), 2.33 (d, *J*=6.5 Hz, 2H), 2.17 (s, 3H), 2.4-2.1 (m, 2H), 2.1-1.4 (m, 10H), 1.26 (m, 1H), 1.2-0.85 (m, 4H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$151.0, 147.7, 138.2, 137.2, 120.9, 92.4, 78.4, 68.4, 56.9, 48.5, 40.9, 40.3, 37.4, 35.1, 32.2, 30.3, 27.0, 22.6;

**8-[5-(1-Methyl-2-pyrrolidinyl)-3-pyridinyl]oct-7-ynamine (SG29).** The reaction mixture was heated for 6h. Purification: column chromatography [$SiO_2$, $CHCl_3$/MeOH/$NH_4OH$, (5:1:0.1)]; yield 0.36g (86%) (based on recovered 5-bromonicotine); IR (film) $\nu_{max}$ 2232 $cm^{-1}$;

$^1$H-NMR (270 MHz, $CDCl_3$) $\delta$8.47 (d, $J$=2 Hz, 1H), 8.38 (d, $J$=2 Hz, 1H), 7.68 (dd, $J$=2, 2 Hz, 1H), 3.21 (ddd, $J$=9.5, 9.5, 2 Hz 1H), 3.08-3.01 (m, 1H), 2.71-2.66 (m, 2H), 2.40 (t, $J$=7 Hz, 2H), 2.34-2.11 (m, 5H), 1.98-1.31 (m, 13H); $^{13}$C-NMR (68 MHz, $CDCl_3$) $\delta$147.2, 143.9, 134.5, 133.5, 117.1, 89.7, 64.7, 53.1, 37.8, 36.6, 31.3, 28.7, 24.8, 24.6, 22.5, 18.8, 15.5;

***trans*-4-[3-(5-(1-Methyl-2-pyrrolidinyl)-2-pyridinyl)-prop-2-ynyl]-cyclohexylmethyl-amine (GK90).** A mixture of 6-bromonicotine (0.47g, 2 mmol), tris(dibenzylideneacetone)-dipalladium [$(dba)_3Pd_2$] (0.018g, 0.02 mmol), 1,3-bis(diphenylphosphino)propane (dppp) (0.032 g, 0.08 mmol) and CuI (0.008g, 0.04mmol) in 15 ml of $Et_3N$ was deoxygenated with $N_2$ and then **GK88** (0.326g, 2.16 mmol) was added. The reaction mixture was heated at 120 °C for 6 h in a sealed vessel. The $Et_3N$ was evaporated *in vacuo*. The residue was dissolved in EtOAc, washed with diluted NaOH solution and extracted with 40 ml of 2*N* HCl. The acidic aqueous phase was extracted with EtOAc (4x20 ml). The aqueous layer was alkalinized with solid NaOH to pH12 and extracted with EtOAc (5x30 ml). The combined organic phases was washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by column chromatography [silica, $CHCl_3$/MeOH saturated with $NH_3$, (15:1)] yielding 0.43 g (71%) of **GK90**; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$8.46 (d, $J$=2 Hz, 1H), 7.62 (dd, $J$=8, 2 Hz, 1H), 7.34 (d, $J$=8 Hz, 1H), 3.23 (m, 1H), 3.07 (app t, 1H), 2.53 (d, $J$=6.5 Hz, 2H), 2.34 (d, $J$=6.5 Hz, 2H), 2.30 (app q, 1H), 2.18 (m, 1H), 2.15 (s, 3H), 2.05-1.45 (m, 8H), 1.35-0.85 (m, 7H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$149.5, 142.7, 137.6, 134.8, 126.7, 89.3, 81.3, 68.6, 56.9, 48.7, 41.0, 40.3, 37.4, 35.1, 32.3, 30.3, 26.9, 22.6;

***trans*-3-(5-(1-Methyl-2-pyrrolidinyl)-3-pyridinyl)acrylic acid methyl ester (GK59).** A mixture of 5-bromonicotine (0.241g, 1 mmol), $Pd(OAc)_2$ (0.011g, 0.05 mmol), tri-*o*-tolylphosphine (0.060 g, 0.2 mmol) and $Et_3N$ (0.17 ml, 1.25 mmol) in 5 ml of MeCN was deoxygenated with $N_2$ and methyl acrylate (0.112 ml, 1.25 mmol) was added. The reaction mixture was heated in a sealed vessel at 110 °C overnight. MeCN was evaporated *in vacuo* and the residue was dissolved in EtOAc, washed with saturated $NaHCO_3$ solution and extracted with 20 ml of 2*N* HCl. The acidic aqueous phase was extracted with EtOAc (4x20 ml). The aqueous layer was saturated with solid $NaHCO_3$ and extracted with EtOAc (3x30 ml). The organic phase was washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by column chromatography [silica, $CHCl_3$/MeOH, (40:1)]; yield 0.2 g (80 %); mp 42-44 °C; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$8.62 (d, $J$=2 Hz, 1H), 8.53 (d, $J$=2 Hz, 1H), 7.87 (t, $J$=2 Hz, 1H), 7.69 (d, $J$=16.0 Hz, 1H), 6.55 (d, $J$=16.0 Hz, 1H), 3.82 (s, 3H), 3.26 (m, 1H), 3.15 (app t, 1H), 2.34 (app q, 1H), 2.22 (m, 1H), 2.18 (s, 3H), 2.1-1.6 (m,

3H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$166.7, 150.9, 148.6, 141.2, 139.3, 132.8, 130.1, 119.9, 68.4, 56.9, 51.8, 40.4, 35.3, 22.6;

**Methyl 4-[(5-(1-methyl-2-pyrrolidinyl)-3-pyridinyl)ethynyl]benzoate (YH19).** A mixture of 5-bromonicotine (0.44 g, 1.82 mmol), $Pd(OAc)_2$ (60 mg, 0.34 mmol), $Ph_3P$ (0.19 g, 0.72 mmol), CuI (30 mg) and $Et_3N$ (30 mL) was stirred at room temperature for 30 min. Methyl 4-ethynylbenzoate[8] (0.32 g, 2 mmol) was added and the mixture was refluxed over night. The reaction mixture was allowed to reach room temperature, diluted with $CH_2Cl_2$ (120 mL) and washed with $H_2O$. The organic phase was dried ($MgSO_4$), filtered and concentrated. The residue was chromatographed [$SiO_2$, $CHCl_3$/MeOH (50:1)] to give 0.37 g (64%) of **YH19**. An analytical sample was recrystallized from EtOH/*iso*-hexane; mp: 97-98 °C; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$8.65 (br s, 1H), 8.49 (br s, 1H), 8.03-7.97 (m, 2H), 7.85 (br s, 1H), 7.59-7.53 (m, 2H), 3.89 (s, 3H), 3.27-3.19 (m, 1H), 3.10 (t, *J*=8.5 Hz, 1H), 2.36-2.13 (m, 2H), 2.17 (s, 3H), 2.04-1.62 (m, 3H); $^{13}$C NMR (270 MHz, $CDCl_3$) $\delta$166.3, 150.9, 148.8, 138.6, 137.4, 131.5, 129.8, 129.5, 127.2, 119.7, 91.5, 89.0, 68.3, 56.9, 52.2, 40.3, 35.2, 22.7;

***General procedure for the hydrolysis of esters (Scheme 6).***

**4-[(5-(1-Methyl-2-pyrrolidinyl)-2-pyridinyl)ethynyl]benzoic acid (GK49).** A mixture of **GK47** (0.185 g, 0.58 mmol) and KOH (0.097 g, 1.73 mmol) in 50 % aqueous MeOH (10 ml) was heated under reflux for 30 min. The reaction mixture was acidified with HOAc to pH 8 and the solvents were evaporated *in vacuo*. The crude product was purified by column chromatography (silica gel, $CHCl_3$/MeOH, gradient of MeOH 10% to 50%). The solvents were evaporated *in vacuo*. The residue was dissolved in $CH_2Cl_2$/EtOH, (95:5), filtered and evaporated *in vacuo* to give 0.17 g (96%) of **GK49**; $^1$H NMR ($CD_3OD$, 270 MHz) $\delta$8.54 (br s, 1H), 8.04 (d, *J*=8.5 Hz, 2H), 7.91 (dd, *J*=8, 2 Hz, 2H), 7.64 (d, *J*=8 Hz, 1H), 7.60 (d, *J*=8.5 Hz, 2H), 3.46 (app t, 1H), 3.36 (m, 1H), 2.55 (app q, 1H), 2.40–2.20 (m, 4H), 2.10–1.80 (m, 3H); $^{13}$C NMR ($CD_3OD$, 68 MHz) $\delta$174.1, 150.8, 143.5, 138.4, 138.2, 137.9, 132.6, 130.8, 128.9, 125.6, 90.7, 90.2, 70.1, 57.9, 40.5, 35.2, 23.4;

**7-(5-(1-Methyl-2-pyrrolidinyl)-2-pyridinyl)hept-6-ynoic acid (GK81).** The compound was purified by preparative TLC [silica, $CHCl_3$/MeOH, (5:1)]; yield 93%; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$11.49 (br s, 1H), 8.40 (br s, 1H), 8.68 (dd, *J*=8, 2 Hz, 1H), 7.31 (d, *J*=8 Hz, 1H), 3.32 (m, 1H), 3.22 (app t, 1H), 2.5-2.1 (m, 9H), 2.1-1.5 (m, 7H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$ 177.1, 149.3, 142.8, 135.6, 135.3, 126.9, 91.1, 80.0, 68.7, 56.3, 39.6, 34.2, 33.9, 27.8, 24.4, 22.1, 19.1;

**7-(5-(1-Methyl-2-pyrrolidinyl)-3-pyridinyl)hept-6-ynoic acid (GK80).** The compound was purified by preparative TLC [silica, $CHCl_3$/MeOH, (5:1)]; yield 79%; $^1$H NMR ($CDCl_3$, 270

MHz) $\delta$12.5 (br s, 1H), 8.47 (br s, 1H), 8.35 (br s, 1H), 7.75 (s, 1H), 3.30 (m, 1H), 3.18 (app t, 1H), 2.5-2.1 (m, 9H), 2.1-1.5 (m, 7H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$176.8, 150.7, 147.1, 138.2, 136.5, 121.3, 93.9, 77.3, 68.5, 56.3, 39.7, 34.1 (2 C's), 28.0, 24.4, 22.2, 19.2;

***trans*-4-[3-(5-(1-Methyl-2-pyrrolidinyl)-2-pyridinyl)prop-2-ynyl]cyclohexanecarboxylic acid (GK83).** The compound was purified by column chromatography [silica, $CHCl_3$/MeOH, (10:1)]; yield 80%; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$10.2 (br s, 1H), 8.59 (s, 1H), 7.81 (d, $J$=8 Hz, 1H), 7.38 (d, $J$=8 Hz, 1H), 3.33 (m, 1H), 3.23 (m, 1H), 2.5-1.2 (m, 20H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$179.4, 148.7, 141.9, 136.9 (br), 136.0, 127.0, 90.6, 80.6, 68.6, 56.7, 43.1, 40.0, 36.2, 34.6, 31.1, 29.1, 26.7, 22.5;

***trans*-4-[3-(5-(1-Methyl-2-pyrrolidinyl)-3-pyridinyl)prop-2-ynyl]cyclohexanecarboxylic acid (GK82).** The compound was purified by preparative TLC [silica, $CHCl_3$/MeOH, (10:1)]; yield 85%; $^1$H NMR ($CD_3OD$, 270 MHz) $\delta$8.48 (br s, 1H), 8.44 (br s, 1H), 7.90 (s, 1H), 3.50 (app t, 1H), 3.40 (m, 1H), 2.60 (app q, 1H), 2.4-1.8 (m, 14H), 1.65-1.35 (m, 3H), 1.18 (m, 2H); $^{13}$C NMR ($CD_3OD$, 68 MHz) $\delta$181.1, 152.2, 148.7, 139.7, 137.7, 123.2, 94.5, 79.0, 70.1, 57.8, 45.3, 40.3, 38.3, 35.0, 33.1, 30.5, 27.7, 23.3;

***trans*-4-[3-(5-(1-Methyl-2-pyrrolidinyl)-3-pyridinyl)propyl]cyclohexanecarboxylic acid (GK95).** The compound was purified by preparative TLC [silica, $CHCl_3$/MeOH, (10:1)]; yield 93%; $^1$H NMR ($CDCl_3$+$C_6D_6$, 270 MHz) $\delta$ 11.89 (br s, 1H), 8.34 (d, $J$=2 Hz, 1H), 8.32 (d, $J$=2 Hz, 1H), 7.63 (app t, 1H), 3.28 (m, 1H), 2.97 (m, 1H), 2.44 (t, $J$=8 Hz, 2H), 2.35-1.35 (m, 14H), 2.05 (s, 3H), 1.15 (m, 3H), 0.85 (m, 2H); $^{13}$C NMR ($CDCl_3$+$C_6D_6$, 68 MHz) $\delta$179.3, 148.5, 146.5, 138.3, 136.6, 135.0, 68.6, 56.2, 43.9, 39.5, 37.0, 36.8, 34.1, 33.2, 32.4, 32.3, 29.2, 28.3, 22.0;

***trans*-3-(5-(1-Methyl-2-pyrrolidinyl)-3-pyridinyl)acrylic acid (GK62).** The compound was purified by column chromatography [silica, $CHCl_3$/EtOH, (1:1)]; yield 82%; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$13.48 (br s, 1H), 8.73 (s, 1H), 8.47 (s, 1H), 8.40 (s, 1H), 7.64 (d, $J$=16 Hz, 1H), 6.76 (d, $J$=16 Hz, 1H), 3.83 (m, 1H), 3.55 (m, 1H), 2.62 (m, 1H), 2.5-2.0 (m, 7H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$169.9, 149.8, 148.8, 138.1, 134.4, 134.0, 131.7, 125.3, 69.0, 55.6, 38.9, 33.4, 21.9;

**4-[(5-(1-Methyl-2-pyrrolidinyl)-3-pyridinyl)ethynyl]benzoic acid (YH20).** KOH (168 mg, 3 mmol) was added to **YH19** (0.32 g, 1 mmol) in MeOH (5 mL). The stirred mixture was refluxed for 2h and then cooled to room temperature. HOAc was added to pH8 and the volatiles were evaporated. The residue was chromatographed [$SiO_2$, $CHCl_3$/MeOH (5:1)] to give 0.19 g (62%) of **YH20**; $^1$H NMR (270MHz, $CDCl_3$) $\delta$11.00 (br s, OH), 8.75 (br s, 1H), 8.44 (m, 2H), 8.10 (d, $J$=8 Hz, 2H), 7.42 (d, $J$=8.5 Hz, 2H), 3.94-3.84 (m, 1H), 3.63-3.52 (m,

1H), 2.69-2.58 (m, 1H), 2.42 (s, 3H), 2.42-1.98 (m, 4H); $^{13}$C NMR (270 MHz, $CDCl_3$) $\delta$ 169.5, 151.6, 148.9, 139.0, 133.8, 132.9, 131.5, 129.6, 125.3, 120.8, 93.0, 87.4, 69.0, 55.5, 38.8, 33.2, 21.7;

**4-(3-(*N*-Methylpyrrolidin-2-yl)pyridin-5-ylethyl)benzoic acid (YH24).** KOH (130 mg, 2.32 mmol) was added to a solution of **YH22** (0.12 g, 0.37mmol), $H_2O$ (3 mL) and 1,4-dioxane (3 mL). After reflux for 2h the pH of the mixture was adjusted to 8 by addition of HOAc. Volatiles were evaporated and the residue was chromatographed [$SiO_2$, $CHCl_3$/MeOH (2:1)] to give 70 mg (61%) of **YH24**; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$8.44 (br s, 2H), 7.92 (s, 1H), 7.88 (d, *J*=8 Hz, 2H), 7.12 (d, *J*=8 Hz, 2H), 3.98-3.91 (m, 1H), 3.75-3.66 (m, 1H), 2.99-2.85 (m, 5H), 2.50 (s, 3H), 2.43-2.31 (m, 1H), 2.27-2.06 (m, 3H); $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$172.9, 151.0, 148.4, 145.9, 139.6, 137.9, 134.1, 130.8 (2C's), 129.5 (3C's), 70.6, 57.2, 39.5, 38.2, 35.4, 33.7, 22.9;

***General procedure for hydrogenation of triple bonds (Scheme 7).***

***trans*-4-[3-(5-(1-Methyl-2-pyrrolidinyl)-2-pyridinyl)propyl]cyclohexanecarboxylic acid (GK84).** **GK83** (0.153 g, 0.47 mmol) in 40 ml of EtOH was hydrogenated at room temperature and atmospheric pressure over 0.1g of 10% palladium on carbon for 1 h. The catalyst was filtered off and washed with EtOH. The solvent was evaporated *in vacuo* and the residue was purified by preparative TLC [silica, $CHCl_3$/MeOH, (10:1)] to give 0.07 g (45%) of **GK84**; $^1$H NMR ($CD_3OD$, 270 MHz) $\delta$8.55 (d, *J*=2 Hz, 1H), 7.96 (dd, *J*=8, 2 Hz, 1H), 7.42 (d, *J*=8 Hz, 1H), 4.14 (m, 1H), 3.69 (m, 1H), 3.08 (m, 1H), 2.81 (t, *J*=7.5 Hz, 2H), 2.61 (s, 3H), 2.47 (m, 1H), 2.21 (m, 4H), 2.0-1.6 (m, 6H), 1.5-1.1 (m, 5H), 0.96 (m, 2H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$180.0, 163.3, 148.8, 136.8, 128.6, 123.7, 69.5, 55.8, 43.6, 38.4, 37.6, 32.3, 32.2, 29.1, 27.0, 21.5;

***trans*-4-[3-(5-(1-Methyl-2-pyrrolidinyl)-3-pyridinyl)-propyl]cyclohexylmethylamine (GK93).** Purification: column chromatography [alumina, $CHCl_3$/MeOH, gradient of methanol 10-50 %]; yield 78%; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$8.34 (d, *J*=2 Hz, 1H), 8.32 (d, *J*=2 Hz, 1H), 7.50 (t, *J*=2 Hz, 1H), 3.25 (m, 1H), 3.06 (app t, 1H), 2.58 (t, *J*=7.5 Hz, 2H), 2.51 (d, *J*=6.5 Hz, 2H), 2.30 (app q, 1H), 2.19 (m, 1H), 2.17 (s, 3H), 2.05-1.55 (m, 9H), 1.32 (br s, 2H), 1.22 (m, 4H), 0.88 (m, 4H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$148.8, 147.0, 138.0, 137.8, 134.3, 68.8, 56.9, 48.8, 41.3, 40.3, 37.6, 36.9, 35.1, 33.2, 32.7, 30.6, 28.5, 22.5;

***trans*-4-[3-(5-(1-Methyl-2-pyrrolidinyl)-3-pyridinyl)propyl]cyclohexanecarboxylic acid methyl ester (GK94).** Purification: column chromatography [silica, acetone/*iso*-hexane (1:2)]; yield 79%; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$8.34 (d, *J*=2 Hz, 1H), 8.31 (d, *J*=2 Hz, 1H), 7.50 (d, *J*=2 Hz, 1H), 3.65 (s, 3H), 3.25 (m, 1H), 3.06 (app t, 1H), 2.58 (t, *J*=8 Hz, 2H), 2.4-

2.1 (m, 3H), 2.17 (s, 3H), 2.05-1.55 (m, 9H), 1.40 (m, 2H), 1.24 (m, 3H), 0.91 (m, 2H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$176.5, 148.8, 147.1, 138.1, 137.7, 134.3, 68.8, 57.0, 51.4, 43.3, 40.4, 36.7, 35.1, 33.2, 32.2, 28.9, 28.4, 22.5;

***trans*-4-[3-(5-(1-Methyl-2-pyrrolidinyl)-2-pyridinyl)-propyl]cyclohexylmethylamine (GK96).** Purification: preparative TLC [silica, $CHCl_3$/MeOH saturated with $NH_3$ (15:1)]; yield 81%; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$8.41 (d, $J$=2 Hz, 1H), 7.60 (dd, $J$=8, 2 Hz, 1H), 7.11 (d, $J$=2 Hz, 1H), 3.23 (m, 1H), 3.04 (app t, 1H), 2.74 (t, $J$=8 Hz, 2H), 2.51 (d, $J$=6.5 Hz, 2H), 2.29 (app q, 1H), 2.19 (m, 1H), 2.16 (s, 3H), 2.05-1.65 (m, 9H), 1.49 (br s, 2H), 1.35-1.10 (m, 4H), 0.88 (m, 4H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$161.4, 148.8, 135.5, 135.1, 122.5, 68.6, 57.0, 48.8, 41.4, 40.3, 38.4, 37.8, 37.1, 35.0, 32.8, 30.6, 27.4, 22.5;

**8-[5-(1-Methyl-2-pyrrolidinyl)-3-pyridinyl]octanamine (SG30).** A solution of **SG29** (0.2 g, 0.7 mmol) in MeOH (20 ml) was hydrogenated at room temperature and at atmospheric pressure over 10% Pd/C (0.1 g). After 15 min the catalyst was filtered off and washed with MeOH. The volatiles was evaporated under reduced pressure and the residue was chromatographed [$SiO_2$, $CHCl_3$/MeOH/$NH_4OH$, (5:1:0.1)] to afford 0.16 g (76%) of **SG30** as a colorless oil. $^1$H NMR (270 MHz, $CDCl_3$) $\delta$8.30 (d, $J$=2 Hz, 1H), 8.28 (d, $J$=2 Hz, 1H), 7.47 (brs, 1H), 3.21 (ddd, $J$=9.5, 9.5, 1.5 Hz, 1H), 2.99-3.05 (m, 1H), 2.64 (t, $J$=7 Hz, 2H), 2.56 (br t, $J$=8 Hz, 2H), 2.27 (dd, $J$=17.5, 9 Hz, 1H), 2.09-2.18 (m, 4H), 1.55-1.94 (m, 7H), 1.27-1.42 (m, 10H); $^{13}$C-NMR (68 MHz, $CDCl_3$) $\delta$149.0, 147.1, 138.2, 138.1, 134.6, 69.0, 57.2, 42.3, 40.6, 35.3, 33.8, 33.1, 31.3, 29.5, 29.3, 27.0, 22.7;

**4-[2-(5-(1-Methyl-2-pyrrolidinyl)-2-pyridinyl)ethyl]benzoic acid (GK54). GK49** (0.09g, 0.29 mmol) in 20 ml of MeOH was hydrogenated at room temperature and atmospheric pressure over 0.03g of 10% palladium on carbon for 6 h. The catalyst was filtered off and washed with MeOH. The solvent was evaporated *in vacuo* and the residue was purified by silica gel chromatography [$CHCl_3$/MeOH, (5:1)] to give 0.052 g (57%) of **GK54**; $^1$H NMR ($CD_3OD$, 270 MHz) $\delta$8.52 (d, $J$=2 Hz, 1H), 7.88 (d, $J$=8.5 Hz, 2H), 7.83 (dd, $J$=8, 2 Hz, 1H), 7.25 (d, $J$=8 Hz, 1H), 7.19 (d, $J$=8.5 Hz, 2H), 3.74 (m, 1H), 3.51 (m, 1H), 3.08 (m, 4H), 2.76 (m, 1H), 2.41 (s, 3H), 2.36 (m, 1H), 2.07 (m, 3H); $^{13}$C NMR ($CD_3OD$, 68 MHz) $\delta$174.2, 162.7, 150.0, 146.1, 138.2, 134.5, 133.1, 130.8, 129.3, 125.1, 70.4, 57.5, 40.2, 39.8, 37.0, 33.9, 23.0;

**Methyl 4-(3-(*N*-methylpyrrolidin-2-yl)pyridin-5-ylethyl)benzoate (YH22).** A mixture of **YH19** (0.15 g, 0.468 mmol) and Pd(C) (10%, 20 mg) in MeOH (10 mL) was hydrogenated at atmospheric pressure and room temperature for one day. The catalyst was removed by filtration through Celite and the filtrate was concentrated. The residue was chromatographed

[$SiO_2$, $CHCl_3$/MeOH (60:1)] to give 0.15 g (95%) of **YH22**; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.38 (br s, 2H), 7.91-7.80 (m, 2H), 7.40 (s, 1H), 7.16-7.14 (m, 2H), 3.85 (s, 3H), 3.23-3.16 (m, 1H), 3.05-2.87 (m, 5H), 2.30-2.22 (m, 1H), 2.18-2.08 (m, 1H), 2.09 (s, 3H), 1.92-1.84 (m, 1H), 1.81-1.72 (m, 1H), 1.67-1.57 (m, 1H); $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$166.9, 148.6, 147.3, 146.2, 138.3, 136.5, 134.5, 129.7, 128.0, 68.6, 56.9, 51.9, 40.2, 37.4, 35.0, 34.4, 22.4;

**2-Ethynyl-5-(1-methyl-2-pyrrolidinyl)pyridine (GK58).** **GK55** (0.3 g, 1.23 mmol) and NaH as a 60 % dispersion in mineral oil (0.01 g, 0.17 mmol) were dissolved in dry toluene (50 ml). The stirred solution was slowly distilled until the boiling point of the distillate reached 110 °C (approx. 25 ml of distillate collected). The rest of the toluene was evaporated *in vacuo*. The residue was chromatographed [$SiO_2$, $CHCl_3$/MeOH, (10:1)] to give 0.2 g, (87%) of **GK58** as a brown oil; $^1$H NMR ($CDCl_3$, 270 MHz) $\delta$8.51 (d, $J$=2 Hz, 1H), 7.68 (dd, $J$=8, 2 Hz, 1H), 7.45 (d, $J$=8 Hz, 1H), 3.24 (m, 1H), 3.14 (s, 1H), 3.11 (app t, 1H), 2.32 (app q, 1H), 2.20 (m, 1H), 2.16 (s, 3H), 2.05–1.62 (m, 3H); $^{13}$C NMR ($CDCl_3$, 68 MHz) $\delta$149.7, 141.0, 139.1, 135.0, 127.3, 82.8, 76.6, 68.5, 56.9, 40.3, 35.2, 22.6;

***Synthesis of GK56 and GK60 (Scheme 8).***

**5-(1-Methyl-2-pyrrolidinyl)-2-pyridinylpropiolic acid (GK60).** A solution of **GK58** (0.175 g, 0.94 mmol) in THF (20 ml) was cooled to –78 °C and BuLi (1.6M solution in hexane, 0.62 ml, 0.99 mmol) was added. The reaction mixture was stirred for 0.5 h at –78 °C and then $CO_2$ gas was added. After an additional 1 h at –78 °C the reaction mixture was allowed to warm to room temperature. THF was evaporated *in vacuo* and the residue was purified by preparative TLC [silica gel, $CHCl_3$/MeOH, (1:1)]; yield 0.20 g, (90%); $^1$H NMR ($CD_3OD$, 270 MHz) $\delta$ 8.49 (dd, $J$=2, 0.5 Hz, 1H), 7.85 (dd, $J$=8, 2 Hz, 1H), 7.59 (dd, $J$=8, 0.5 Hz, 1H), 3.34-3.21 (m, 2H), 2.42 (app q, 1H), 2.29 (m, 1H), 2.20 (s, 3H), 2.05–1.70 (m, 3H); $^{13}$C NMR ($CD_3OD$, 68 MHz) $\delta$160.4, 150.6, 142.5, 139.8, 137.7, 129.2, 87.4, 78.0, 70.0, 58.0, 40.7, 35.7, 23.5;

**5-(1-Methyl-2-pyrrolidinyl)-3-pyridinylpropiolic acid (GK56)** The compound was synthesized from 3-ethynyl-5-(1-methyl-2-pyrrolidinyl)pyridine[7] following the method described for the synthesis of **GK60** to give **GK56** in a 80% yield after purification by preparative TLC [silica gel, $CHCl_3$/MeOH, (1:1)]; $^1$H NMR ($CD_3OD$, 270 MHz) $\delta$8.56 (br s, 1H), 8.50 (br s, 1H), 7.93 (t, $J$=2 Hz, 1H), 3.22 (m, 2H), 2.38 (app q, 1H), 2.26 (m, 1H), 2.18 (s, 3H), 2.10–1.6 (m, 3H); $^{13}$C NMR ($CD_3OD$, 68 MHz) $\delta$160.7, 152.1, 149.7, 140.4, 140.2, 121.2, 91.0, 75.9, 69.7, 58.0, 40.7, 36.0, 23.6;

## III. Preparation of various nicotine immunogens (nicotine-linker-carrier protein) (Scheme 9).

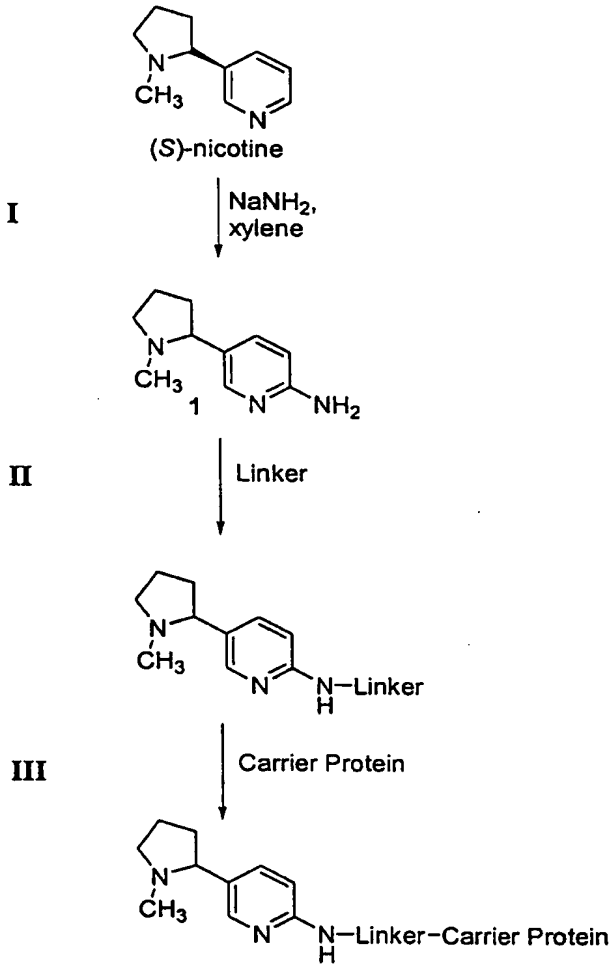
***General procedure for the coupling of carboxylic acids to Keyhole Limpet Hemocyanin (KLH).***

A solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (9 equiv) and distilled $H_2O$ (500 μL) was added to a mixture of the acid (1 equiv) in distilled $H_2O$ (500 μL) at 0 °C. After 10 min a mixture of KLH (the same amount (mg) as of the acid) in distilled $H_2O$ (1 mL) was added. The reaction mixture was kept at 0 °C for 10 min and then at ambient temperature over night. The pH of the reaction mixture was maintained at pH 4.5-6 during the reaction. The protein conjugate was purified by column chromatography [Sephadex G-25M, (PD-10 column), eluted with distilled $H_2O$] and then freeze-dried.

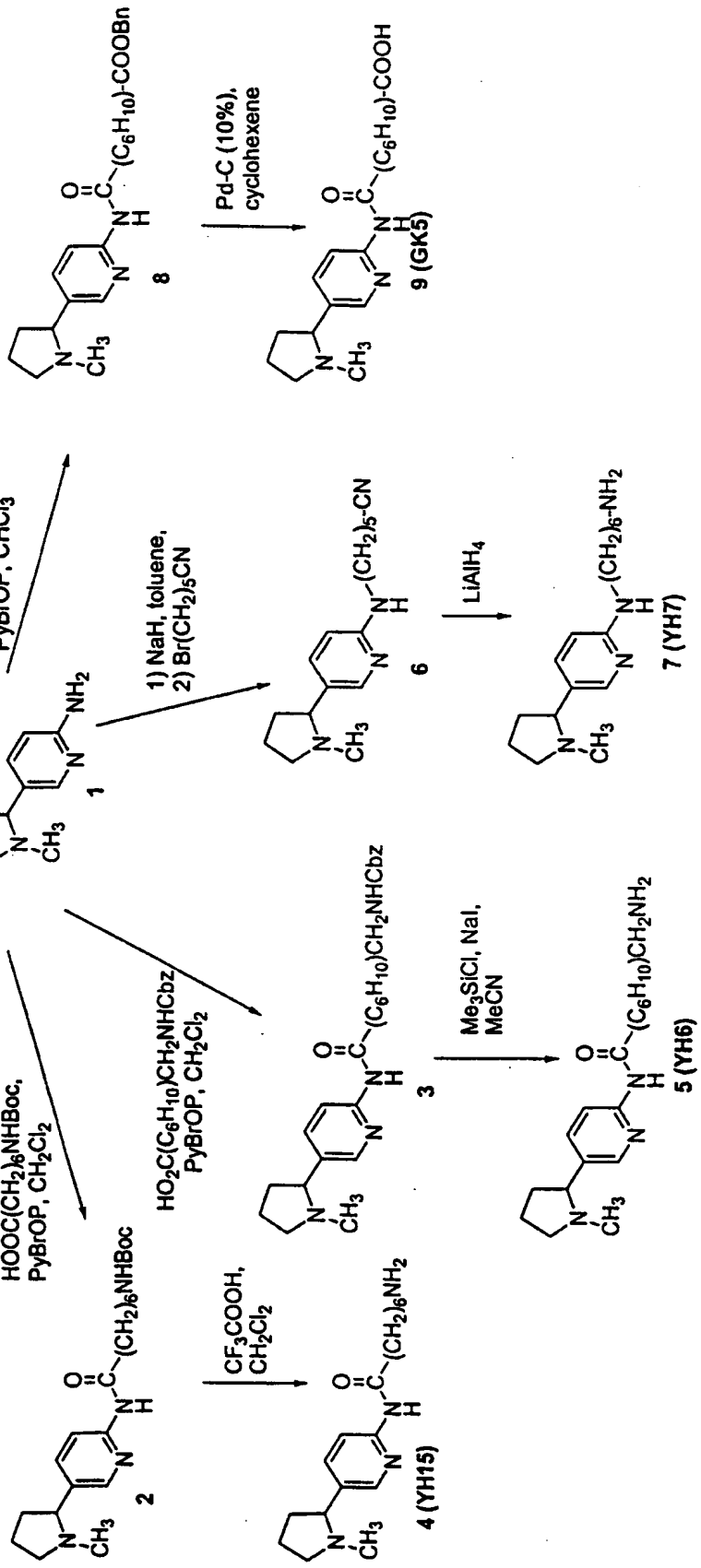
***General procedure for the coupling of amines to KLH.***

A mixture of KLH (the same amount (mg) as of the amine) and $H_2O$ (1 mL) was added to a mixture of the amine (10 mg) in $H_2O$ (500 μL) at 0 °C. A solution of EDC (9 equiv) in $H_2O$ (500 μL) was added to the reaction mixture and the mixture was kept at 0 °C for 10 min and at ambient temperature over night. The pH of the reaction mixture was maintained at pH 4.5-6 during the reaction. The protein conjugate was purified by column chromatography [Sephadex G-25M, (PD-10 column), eluted with distilled $H_2O$] and then freeze-dried.

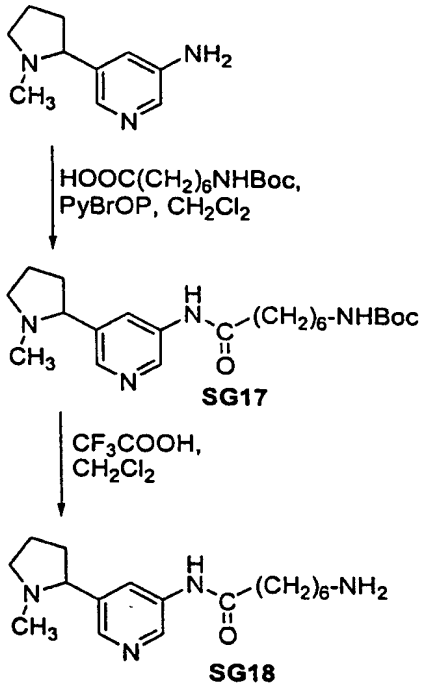
**Scheme 1. Preparation of nicotine immunogens**



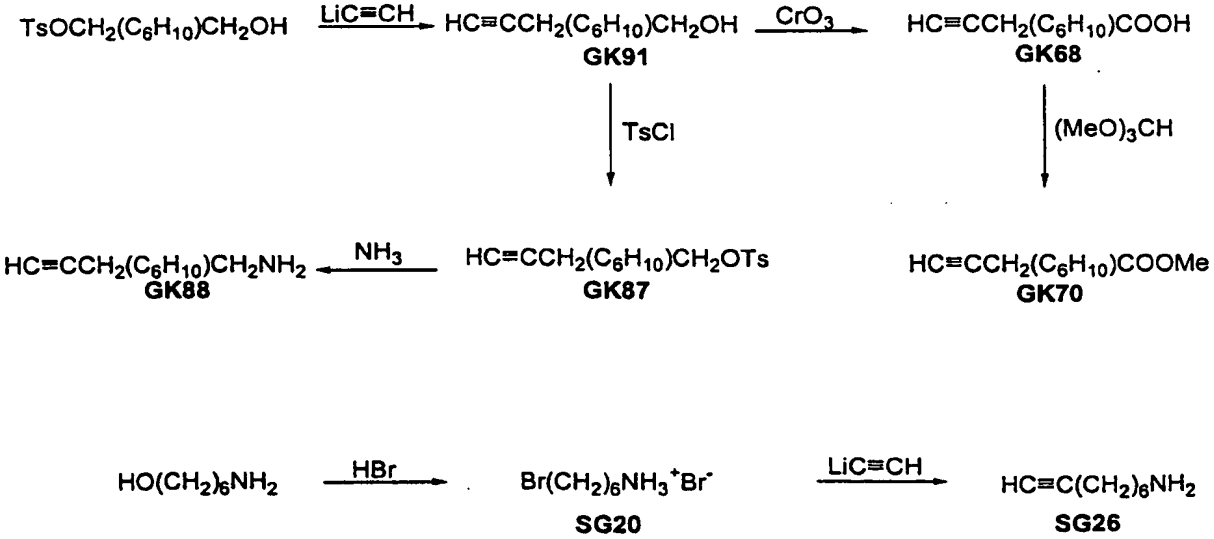
**Scheme 2. Synthesis of 6-aminonicotine-linker molecules.**



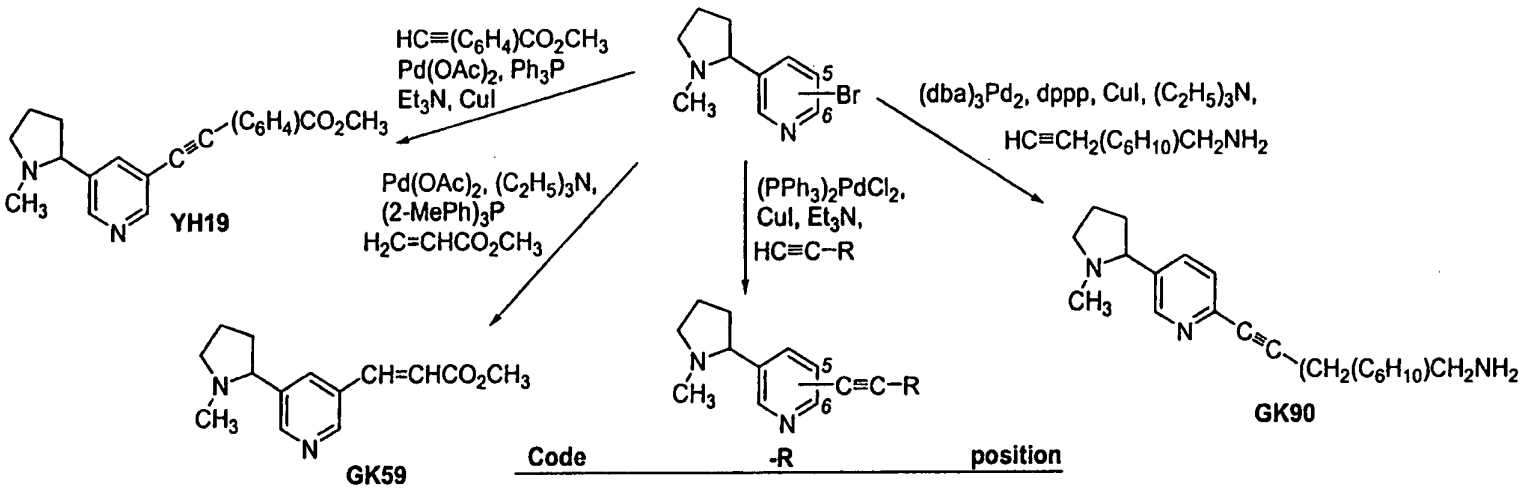
## Schem 3. Synthesis of a 5-aminonicotine derivative.

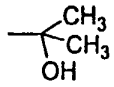



## Scheme 4. Synthesis of some linkers



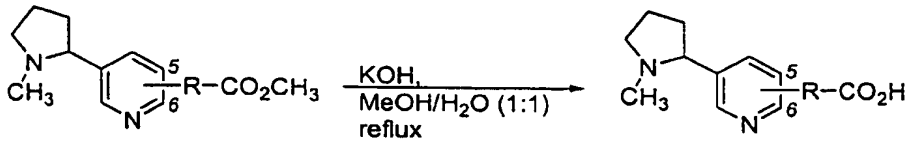
## Scheme 5. Palladium catalyzed coupling reactions of unsaturated derivatives to 5- or 6-bromonicotine.




| Code | -R | position |
|---|---|---|
| GK47 | —$(C_6H_4)$-$CO_2CH_3$ | 6 |
| GK79 | —$(CH_2)_4$-$CO_2CH_3$ | 6 |
| GK77 | —$(CH_2)_4$-$CO_2CH_3$ | 5 |
| GK78 | —$CH_2(C_6H_{10})$-$CO_2CH_3$ | 6 |
| GK75 | —$CH_2(C_6H_{10})$-$CO_2CH_3$ | 5 |
| GK89 | —$CH_2(C_6H_{10})CH_2NH_2$ | 5 |
| GK55 |  | 6 |
| SG29 | —$(CH_2)_6$-$NH_2$ | 5 |

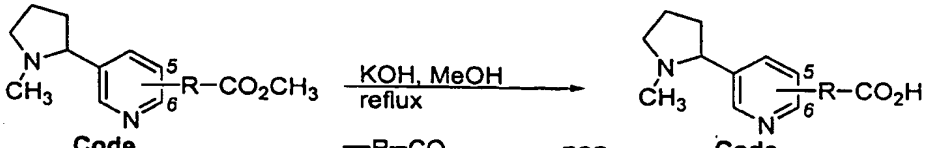
## Sch me 6. Hydrolysis of esters.

I.




| Code | —R—$CO_2$— | pos. | Code |
|---|---|---|---|
| GK47 | —C≡C($C_6H_4$)-$CO_2$- | 6 | GK49 |
| GK79 | —C≡C($CH_2$)$_4$-$CO_2$- | 6 | GK81 |
| GK77 | —C≡C($CH_2$)$_4$-$CO_2$- | 5 | GK80 |
| GK78 | —C≡C$CH_2$($C_6H_{10}$)-$CO_2$- | 6 | GK83 |
| GK75 | —C≡C$CH_2$($C_6H_{10}$)-$CO_2$- | 5 | GK82 |
| GK94 | —($CH_2$)$_3$($C_6H_{10}$)-$CO_2$- | 5 | GK95 |
| GK59 | —CH=CH-$CO_2$- | 5 | GK62 |

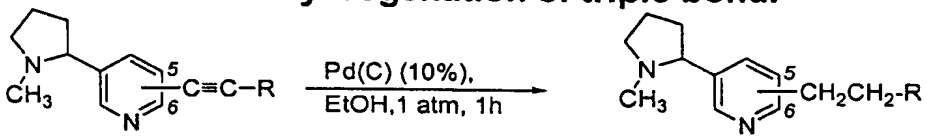
II.




| Code | —R—$CO_2$- | pos. | Code |
|---|---|---|---|
| YH19 | —C≡C($C_6H_4$)-$CO_2$- | 5 | YH20 |
| YH22 | —($CH_2$)$_2$($C_6H_4$)-$CO_2$- | 5 | YH24 |

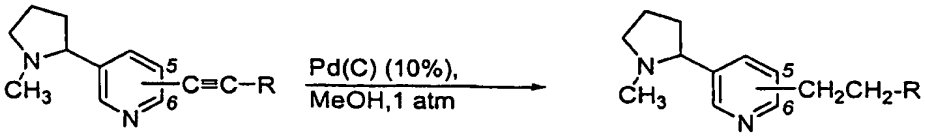
## Scheme 7. Hydrogenation of triple bond.

I.



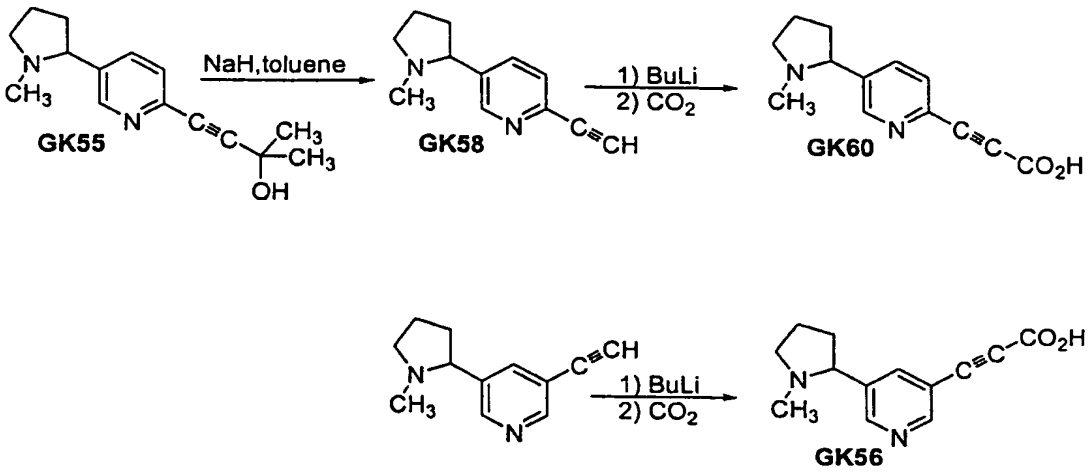

| Code | —R | pos. | Code |
|---|---|---|---|
| GK83 | —$CH_2$($C_6H_{10}$)-$CO_2H$ | 6 | GK84 |
| GK89 | —$CH_2$($C_6H_{10}$)-$CH_2NH_2$ | 5 | GK93 |
| GK75 | —$CH_2$($C_6H_{10}$)-$CO_2CH_3$ | 5 | GK94 |
| GK90 | —$CH_2$($C_6H_{10}$)-$CH_2NH_2$ | 5 | GK96 |

II.

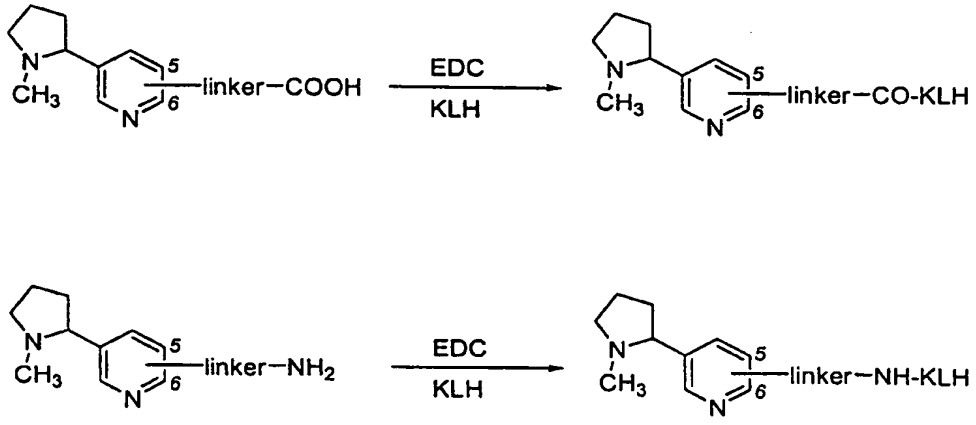



| Code | —R | pos. | Code |
|---|---|---|---|
| SG29 | —($CH_2$)$_6$-$NH_2$ | 5 | SG30 |
| GK49 | —($C_6H_4$)-$CO_2H$ | 6 | GK54 |
| YH19 | —($C_6H_4$)-$CO_2CH_3$ | 5 | YH22 |

## Scheme 8. Synthesis of GK56 and GK60.




## Scheme 9. Synthesis of nicotine-linker-KLH derivatives (nicotine immunogens).



**Description of experiments**

MATERIALS AND METHODS

**Animals**

Male Wistar rats were housed in groups of 2-3 under standard laboratory conditions and maintained under a 12 hr light and dark cycle (lights on at 0600 hr) with unlimited access to food and water.

**Immunization procedures**

Three immunization protocols were used:

1. Animals were immunized daily for five days, then rested for two days, and again immunized daily for five days. Each immunization injection contained 10 μg antigen (nicotine-linker-KLH conjugate) in 0.1 ml saline and was administered subcutaneously (s.c.) in the posterior neck region of the body. Controls were injected with either 10 μg KLH in 0.1 ml saline or 0.1 ml saline alone.
2. Animals were immunized using 100 μg antigen (nicotine-linker-KLH conjugate) and Freund's complete adjuvant in a bolus injection and then given a booster injection on day 11 or 14, containing 100 μg antigen (nicotine-linker-KLH conjugate) and Freund's incomplete adjuvant; these injections were given i.p in 0.4 ml. Controls were injected with either 100 μg KLH in Freund's complete adjuvant or Freund's complete adjuvant alone. In the booster injections Freund's incomplete adjuvant was used in stead of Freund's complete adjuvant.
3. Nicotine self-administering animals were immunized using 100 μg antigen (nicotine-linker-KLH conjugate) and Freund's complete adjuvant in a bolus injection and then given a booster injection on day 7 or 8, containing 100 μg antigen (nicotine-linker-KLH conjugate) and Freund's incomplete adjuvant; these injections were given i.p in 0.4 ml.

**Elisa**

Elisa-plates (Sigma or Labsystems) were coated with a nicotine-linker-BSA conjugate. When required the plates were blocked using a solution of 3% BSA in PBS. After extensive wash, serum was added in various dilutions (1:1 to 1:390625) and the plates incubated at 37°C. The plates were washed again and after adding the secondary antibody, an alkaline phosphatase conjugated goat anti-rat IgG (Sigma), they were further incubated at 37°C. The enzyme substrate *p*-nitrophenyl phosphate (*p*-NPP) (Sigma) produces a coloured end product that can be read spectro-photometrically at 405 nm.

**Competitive Elisa**

The competitive Elisa was performed as the ordinary Elisa with the exception that only a single dilution (1:3125) of serum was used. Nicotine, cotinine, nornicotine, nicotine-N-oxide and niacin respectively were used as competitors.

**Electrophysiological single-cell recording of dopamine neurons in the VTA**

Rats were anaesthetised using chloral hydrate (400 mg/kg, i.p.) with additional doses given when needed to maintain surgical anaesthesia throughout the experiment. Body temperature was maintained at 37°C using a thermostatically controlled heating pad. A tracheal cannula and a jugular vein catheter were inserted before the animal was mounted in a David Kopf stereotaxic instrument. A hole was drilled above the recording area, i.e., AP +3.0, measured from lambda, and ML ± 0.7 mm (Paxinos and Watson, 1986). Electrodes were pulled from Omegadot glass capillaries and filled with Pontamine Sky Blue (2%) in 2 M sodium acetate. The tips were broken under microscope, yielding an impedance of approximately 2.0 MΩ measured at 135 Hz. Presumed DA neurones were found 7.5-8.5 mm from brain surface with characteristics of previously histochemically identified DA neurones (Grace and Bunney, 1983; Wang, 1981), i.e., typical triphasic spike waveforms of more than 2.0 ms duration and basal firing rates of 1-10 Hz. Extracellular action potentials were amplified, discriminated and monitored on an oscilloscope and an audiomonitor. Discriminated spikes were fed, via a Cambridge Electronics design 1401 interface, into an AST Bravo LC 4/66d computer with Spike 2 software. Analysis of the temporal pattern of firing of the DA neurones was performed off-line with a custom analysis script developed in our laboratory. Burst firing, firing rate, and variation coefficient were calculated over a period of 250 consecutive inter-spike time intervals. The onset of a burst was defined as an interval shorter than 80 ms and burst termination at the next interval exceeding 160 ms (Grace and Bunney, 1984). Burst firing was quantified as the percentage ratio between spikes in bursts and the total number of spikes. Variation coefficient was defined as the percentage ratio between the standard deviation and the mean value of the inter-spike intervals (Werner and Mountcastle, 1963).

Nicotine was administered i.v. in the dose range 3-100 μg/kg, cumulative dose, with doubling of the dose for each step. Nicotine injections were given every 3-5 min. At the end of each experiment a negative current of 5 μA was passed for 10 min through the electrode to mark the recording site (Lodge et al., 1974). The animals were killed by an overdose of chloral hydrate and the brains were preserved in 5% formaldehyde in 25%

sucrose and later sliced on a microtome in 50 μm thick sections and stained with neutral red for histological verification of recording sites. Only recording sites located within the VTA were accepted in this protocol.

**Voltammetry**

Voltammetric measurements of dopamine were performed in rats pre-treated with pargyline and kept under chloral hydrate anaesthesia. The active part of the carbon fibre electrode was 12 μm thick and 500 μm long. Electrodes were prepared and treated as described by Gonon (1988). Electrodes were positioned at the coordinates: AP= +1.6 and ML= ±0.8 for the $NAC_{shell}$ (Paxinos and Watson, 1986). The tip of the electrode was placed 6.5-7.0 mm below cortical surface and differential normal pulse voltammetry was used to record voltammograms every min with parameters described previously (Gonon et al., 1984 and Gonon, 1988). When a stable baseline was observed the animal was injected iv. with saline and thereafter nicotine in increasing doses (6, 12, 24 and 48 μg/kg) administered at 10 min intervals.

**Examples**

At present, 16 different nicotine immunogens have been tested for immunogenicity using both *in vitro* and *in vivo* methods.

**Example 1**

**Elisa**

Rats immunized with GK5-KLH conjugate according to protocol 2 were used. Blood samples for Elisa experiments were collected 7 or 9 days after the first bolus immunization. The Elisa antibody titers ranged between 1:100 and 1:15000, see Figure 1.

Blood samples were again collected on day 1-4 post-booster immunization. This second immunisation gave titers between 1:3000 to 1:15 500, see Figure 2.

**Example 2**

**Elisa**

After immunization with GK5-KLH using immunization protocol 1, serum from experimental animals were collected and the specific antibody titres were typically 1:500-1:3000 measured with Elisa technique. Immunization protocol 2, which included an adjuvant, resulted in a higher titre of specific antibodies, typically 1:3000-1:15 000, see figure 3.. All tested nicotine immunogens, e.g. GK5-KLH, GK60-KLH, YH6-KLH, GK84-KLH and GK80-KLH, were immunized using protocol 2. The titres were stable for at least one month.

**Example 3**

**Competitive Elisa**

Competitive Elisa was performed on the serum samples from immunized rats. Nicotine, the metabolites cotinine, nornicotine, nicotine-N-oxide and niacin (not metabolite) were used as competitors. The competitor concentration ranged from $6x10^{-10}$ to $6x10^{-6}$ M. After immunization using protocol 2 immunogens gave rise to antibodies that were more specific to nicotine than to cotinine, nicotine-N-oxide and niacin, see figure 4. In most cases the specificity for nicotine and nornicotine were similar. Cotinine is the major nicotinic metabolite (80%) (Benowitz *et al.* 1994) therefor it is important that the nicotinic antibodies have a greater specificity towards nicotine than cotinine, on the other hand only 0.4 % of the nicotine is metabolised into nornicotine (Benowitz *et al.* 1994) whereas the similarity in specificity towards nicotine and nornicotine should not play a significant role.

**Example 4**

**Electrophysiology**

Electrophysiological single-cell recordings from rat ventral tegmental area dopamine neurones revealed that when nicotine was administered intravenously (3-48 μg/kg), dopamine cells in control (KLH immunized) rats generally responded with an increase in average firing rate and burst firing (see figure 5), as well as a deregularization of the firing pattern, as assessed by the variation coefficient. Five out of six rats tested responded to nicotine. The changes were already evident in doses of nicotine as low as 6 μg/kg. In three out of six rats, the activation was accompanied by a transient (i.e. <1 min) but pronounced increase in neuronal activity immediately following drug administration. Out of these, two cells which did not display bursts before nicotine were converted to a bursty pattern after nicotine administration (6 μg/kg).

In contrast, only one out of four rats immunized with GK5-KLH using immunization protocol 1 responded to nicotine with an increase in dopamine neuronal activity. In addition, transient increases in neuronal activity were not observed, neither was conversion of a non-bursty firing pattern to a bursty one, even when very high doses of nicotine were administered (see figure 6).

Generally, immunized rats thus appear less sensitive to the activating effects of nicotine on ventral tegmental area dopamine neuronal activity. Specifically, immunized rats were not activated by low doses (i.e. < 12 μg/kg) of nicotine like non-immunized rats, and

cells did not either respond with an immediate activation in response to systemic injections of nicotine, as was generally seen in control animals.

In conclusion, these experiments demonstrate that the immunogen used is effective in largely preventing the acute stimulation of burst firing in the mesolimbic dopamine neurons after administration of small nicotine doses equivalent to those ingested by smoking one or two cigarettes. Since the acute effect of e.g. a puff on the brain's reward systems in all probability represents the reinforcing unit in tobacco smoking, this compound looks indeed promising.

**Example 5**

**Voltammetry**

In the controls nicotine administration gives rise to a dose dependent increase of DA-output in the nucleus accumbens shell. In contrast active immunization alters the nicotinic effect on the DA-overflow, see figures 7 a, b and c. Depending on the properties of the immunogen, i.e. the nicotinic hapten, the effect of nicotine on the dopamine output can be suppressed to baseline levels (figure 7a) or instead even paradoxically increased (figure 7b). In studies (figure 7c) where the immunization occurred already 3 weeks before the actual experiment with nicotine, animals still displayed a marked reduction in the central effect of nicotine even in high doses on the mesolimbic dopamine system, i.e. the primary reward pathway in the brain, which has proven critical for self administration of nicotine and its dependence liability.

**References in the description of experiments**

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**Lodge D., Caddy K. W. T., Headley P. M., and Biscoe T. J.:** The locations of neurones with pontamine sky blue. Neuropharmacology 13:481-485, 1974.

**Paxinos G. and Watson C.:** The rat brain in stereotaxic coordinates, 2nd ed. Academic Press, London, 1986.

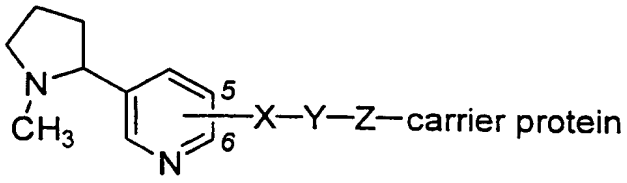
**Wang R. Y.:** Dopaminergic neurones in the rat ventral tegmental area. I. Identification and characterization. Brain Res Rev 3:123-140, 1981.

**Werner G. and Mountcastle V. B.:** The variability of central neuronal activity in a sensory system, and its implications for the central reflection of sensory events. J Neurophysiol 26:958-977, 1963.

29773/BN

## Claims

1. A nicotine immunogen comprising a 5- or 6-nicotinyl- linker - carrier protein having the formula




wherein

X is -NH-CO- or -NH- or -C≡C- or -C=C- or -$CH_2$- ;

Y is -$(CH_2)_k$- or -$(CH_2)_m$-$C_6H_{10}$-$(CH_2)_n$- or -$(CH_2)_m$-$C_6H_4$-$(CH_2)_n$-

wherein k = 0-20, m = 0 - 6, and n = 0 – 6, when

Z is -NH-,

with the provisio that X is not –NH-CO- when Y is $(CH_2)_5$ and Z is -NH-,

and

X is -NH-CO- or -C≡C- or -C=C- or -$CH_2$-,

Y is -$(CH_2)_m$-$C_6H_{10}$-$(CH_2)_n$- or -$(CH_2)_m$-$C_6H_4$-$(CH_2)_n$-

wherein m = 0 - 6, and n = 0 – 6, when

Z is -CO-,

and

X is -C≡C- or -C=C- ,

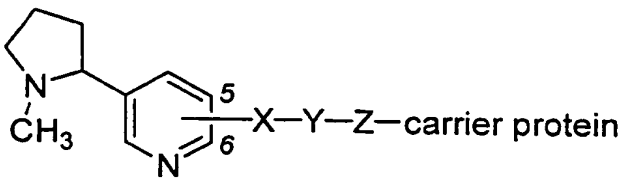
Z is -CO-, when

Y is -$(CH_2)_k$-

wherein k = 0 – 20.

2. The nicotine immunogen according to claim 1, wherein the carrier protein is selected from the group consisting of keyhole limpet hemocyanin (KLH), tetanus toxoid, diphtheria toxoid, non-toxic mutant diphtheria toxoid $CRM_{197}$, outer membrane protein complex (OMPC) from Neisseria meningitidis, the B subunit of heat-labile Escherichia coli, and recombinant exoprotein A from Pseudomonas aeruginosa (rEPA).

3. A 5- or 6 - nicotinyl -linker - carrier protein having the formula




wherein

X is -NH-CO- or -NH- or -C≡C- or -C=C- or -$CH_2$- ;

Y is -$(CH_2)_k$- or -$(CH_2)_m$-$C_6H_{10}$-$(CH_2)_n$- or -$(CH_2)_m$-$C_6H_4$-$(CH_2)_n$-

wherein k = 0-20, m = 0 - 6, and n = 0 – 6, when

Z is -NH-,

with the provisio that X is not –NH-CO- when Y is $(CH_2)_5$ and Z is -NH-,

and

X is -NH-CO- or -C≡C- or -C=C- or -$CH_2$-,

Y is -$(CH_2)_m$-$C_6H_{10}$-$(CH_2)_n$- or -$(CH_2)_m$-$C_6H_4$-$(CH_2)_n$-

wherein m = 0 - 6, and n = 0 – 6, when

Z is -CO-,

and

X is -C≡C- or -C=C- ,

Z is -CO-, when

Y is -$(CH_2)_k$-

wherein k = 0 – 20.

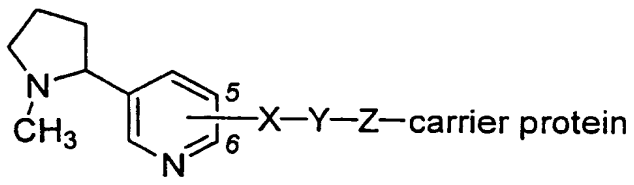
4. A 5- or 6 - nicotinyl -linker - carrier protein according to claim 3, wherein the carrier protein is selected from the group consisting of keyhole limpet hemocyanin (KLH), tetanus toxoid, diphtheria toxoid, non-toxic mutant diphtheria toxoid $CRM_{197}$, outer membrane protein complex (OMPC) from Neisseria meningitidis, the B subunit of heat-labile Escherichia coli, and recombinant exoprotein A from Pseudomonas aeruginosa (rEPA).

5. A 5- or 6 - nicotinyl -linker - carrier protein according to claim 3 or 4 for use as a medicament.

6. Pharmaceutical composition comprising a 5- or 6 - nicotinyl -linker - carrier protein according to claim 4 or 5 and a pharmaceutically acceptable vehicle.

7. Pharmaceutical composition according to claim 6 further comprising an adjuvant.

8. A method of prophylactic and/or therapeutic immunological treatment of nicotine dependence from tobacco products to achieve harm reduction in an individual comprising administration of a 5- or 6 - nicotinyl -linker - carrier protein having the formula




wherein

X is -NH-CO- or -NH- or -C≡C- or -C=C- or $-CH_2-$ ;

Y is $-(CH_2)_k-$ or $-(CH_2)_m-C_6H_{10}-(CH_2)_n-$ or $-(CH_2)_m-C_6H_4-(CH_2)_n-$

wherein k = 0-20, m = 0 - 6, and n = 0 – 6, when

Z is -NH-,

with the provisio that X is not –NH-CO- when Y is $(CH_2)_5$ and Z is -NH-,

and

X is -NH-CO- or -C≡C- or -C=C- or $-CH_2-$,

Y is $-(CH_2)_m-C_6H_{10}-(CH_2)_n-$ or $-(CH_2)_m-C_6H_4-(CH_2)_n-$

wherein m = 0 - 6, and n = 0 – 6, when

Z is -CO-,

and

X is -C≡C- or -C=C- ,

Z is -CO-, when

Y is $-(CH_2)_k-$

wherein k = 0 – 20,

to said individual in antibody-eliciting amounts for eliciting antibodies binding to nicotine molecules.

**9.** The method of treating nicotine dependence according to claim 8, wherein the administration is repeated at intervals to enhance the titre of antibodies binding to nicotine molecules in said individual.

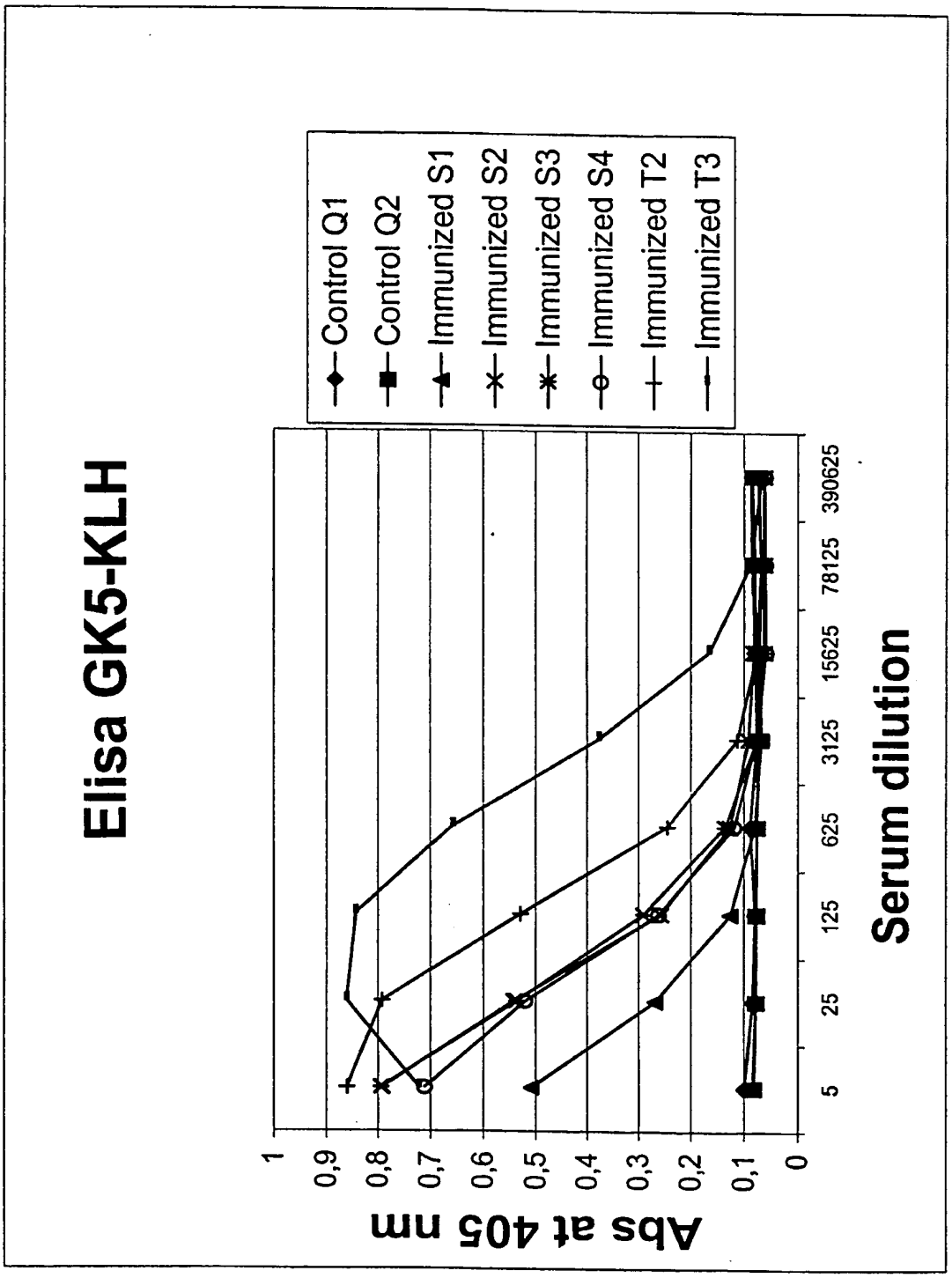

Elisa GK5-KLH
Abs at 405 nm
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
5
25
125
625
3125
15625
78125
390625
Serum dilution
Control Q1
Control Q2
Immunized S1
Immunized S2
Immunized S3
Immunized S4
Immunized T2
Immunized T3

Fig. 1

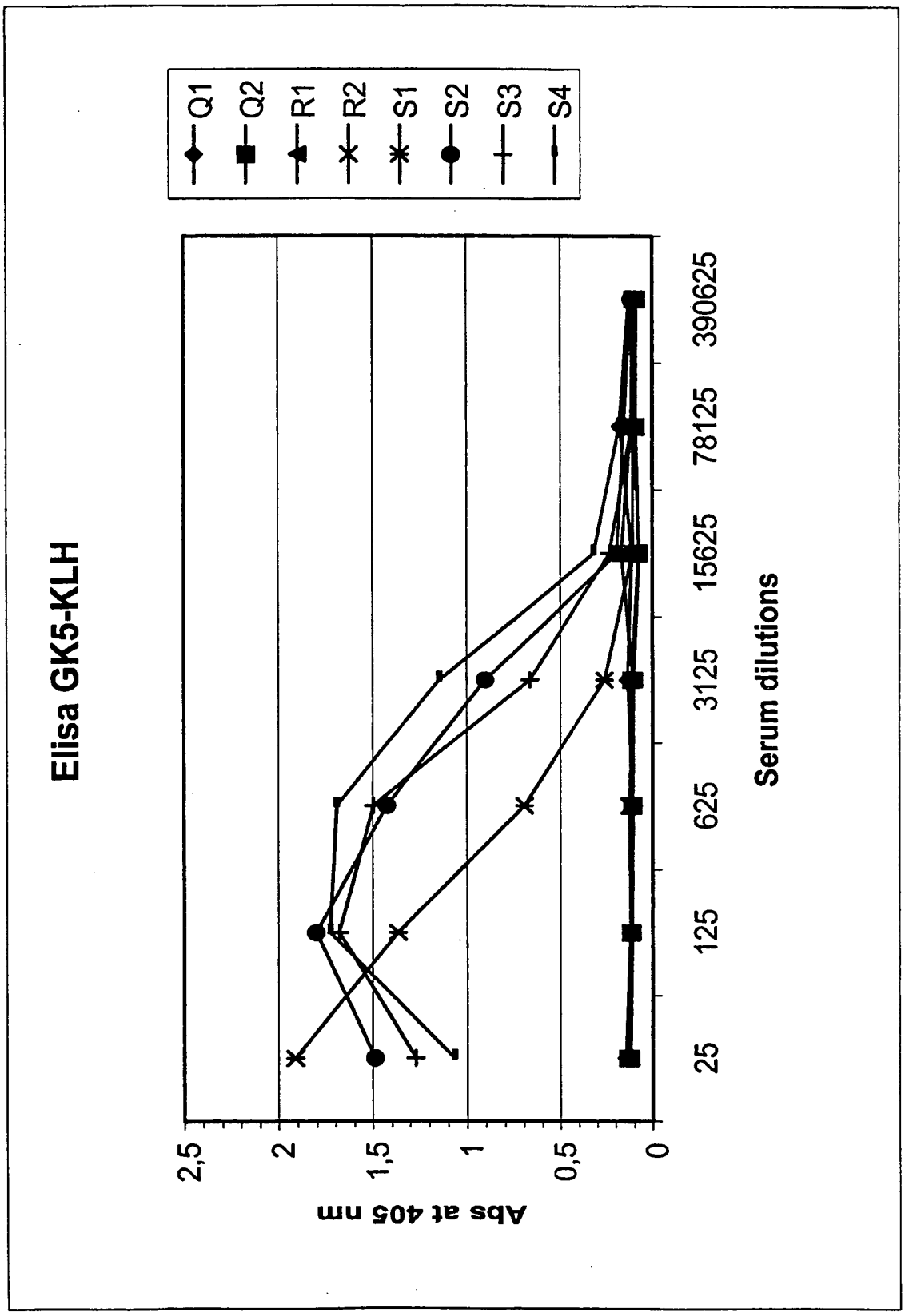

Elisa GK5-KLH
Q1
Q2
R1
R2
S1
S2
S3
S4
Abs at 405 nm
2,5
2
1,5
1
0,5
0
Serum dilutions
25
125
625
3125
15625
78125
390625

Fig. 2

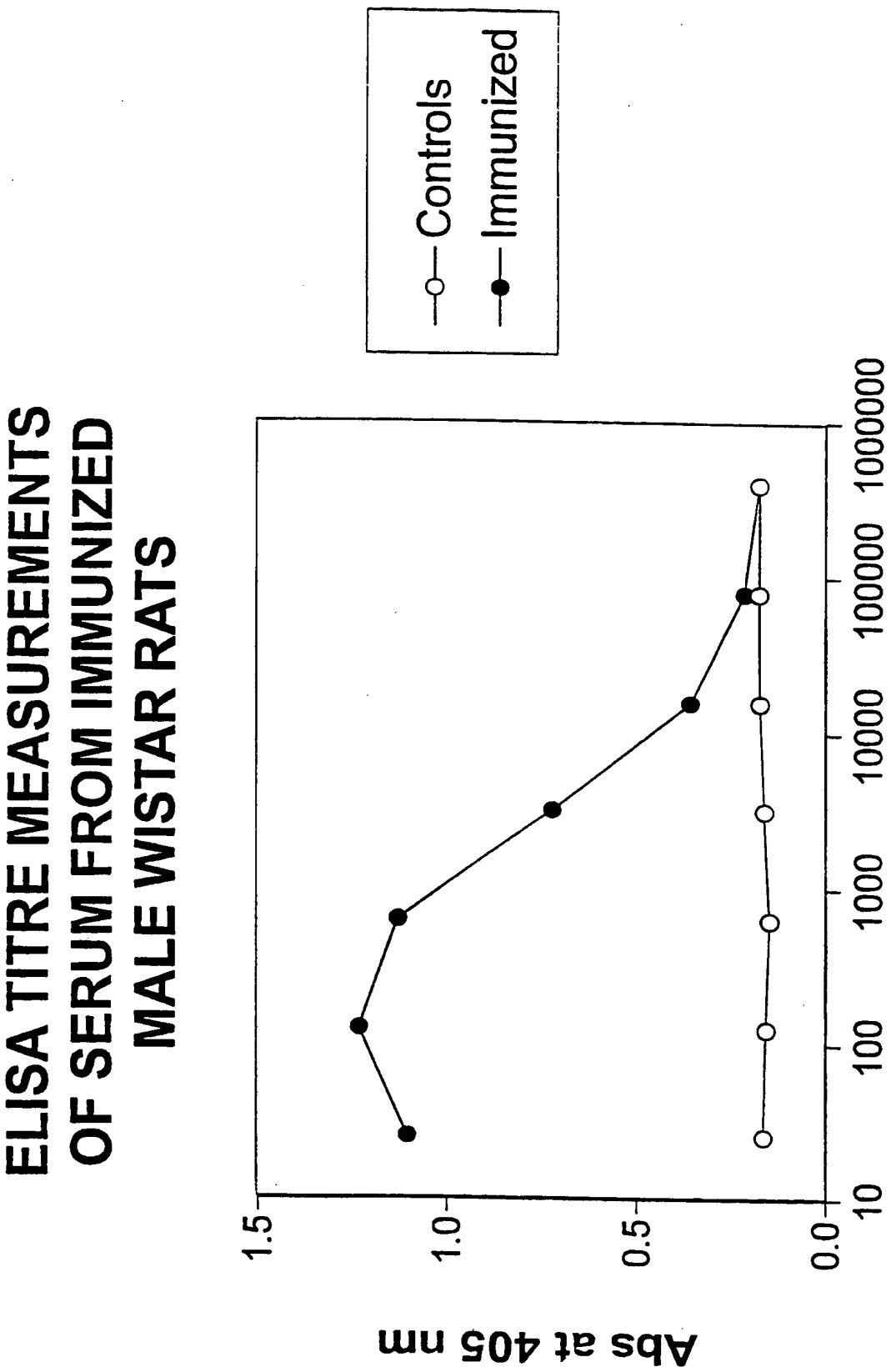

ELISA TITRE MEASUREMENTS
OF SERUM FROM IMMUNIZED
MALE WISTAR RATS
Controls
Immunized
Abs at 405 nm
1.5
1.0
0.5
0.0
10
100
1000
10000
100000
1000000
Serum Dilution

Fig. 3

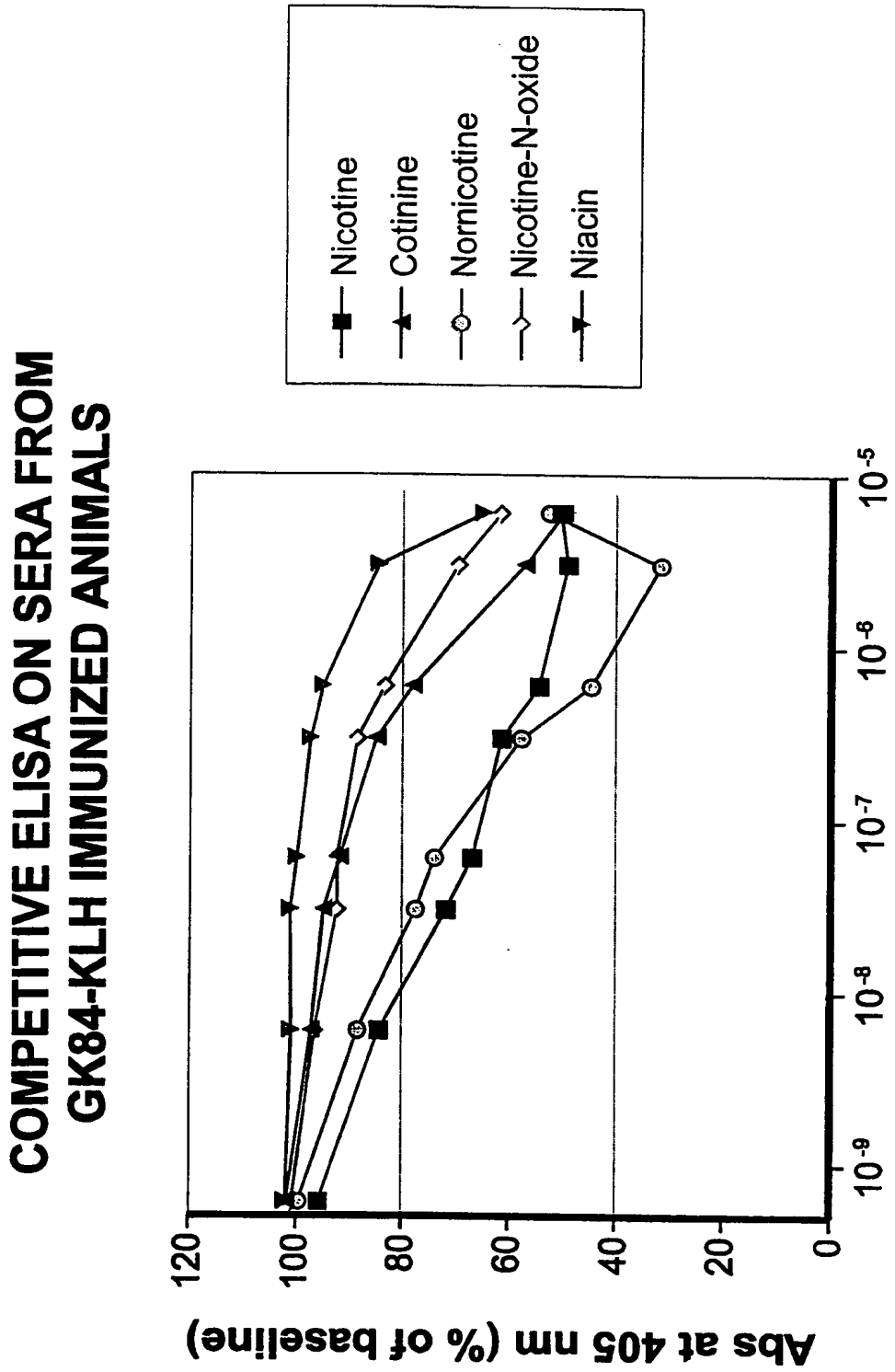

COMPETITIVE ELISA ON SERA FROM
GK84-KLH IMMUNIZED ANIMALS
Nicotine
Cotinine
Normicotine
Nicotine-N-oxide
Niacin
Abs at 405 nm (% of baseline)
120
100
80
60
40
20
0
10-9
10-8
10-7
10-6
10-5
Competitor concentration (M)

Fig. 4

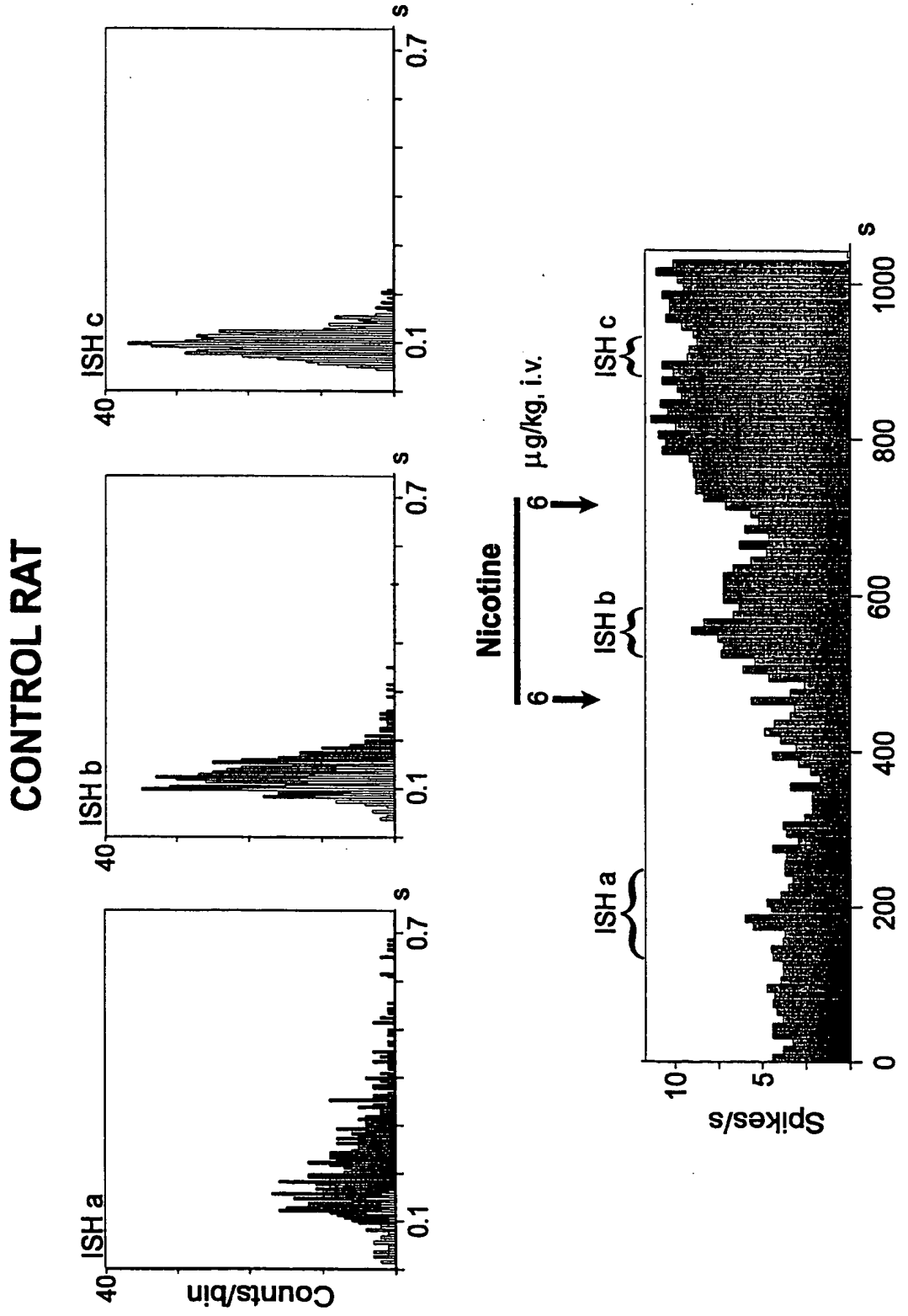

CONTROL RAT
ISH a
ISH b
ISH c
Counts/bin
40
0.1
0.7
s
Nicotine
6
6
µg/kg, i.v.
Spikes/s
10
5
0
200
400
600
800
1000

Fig. 5

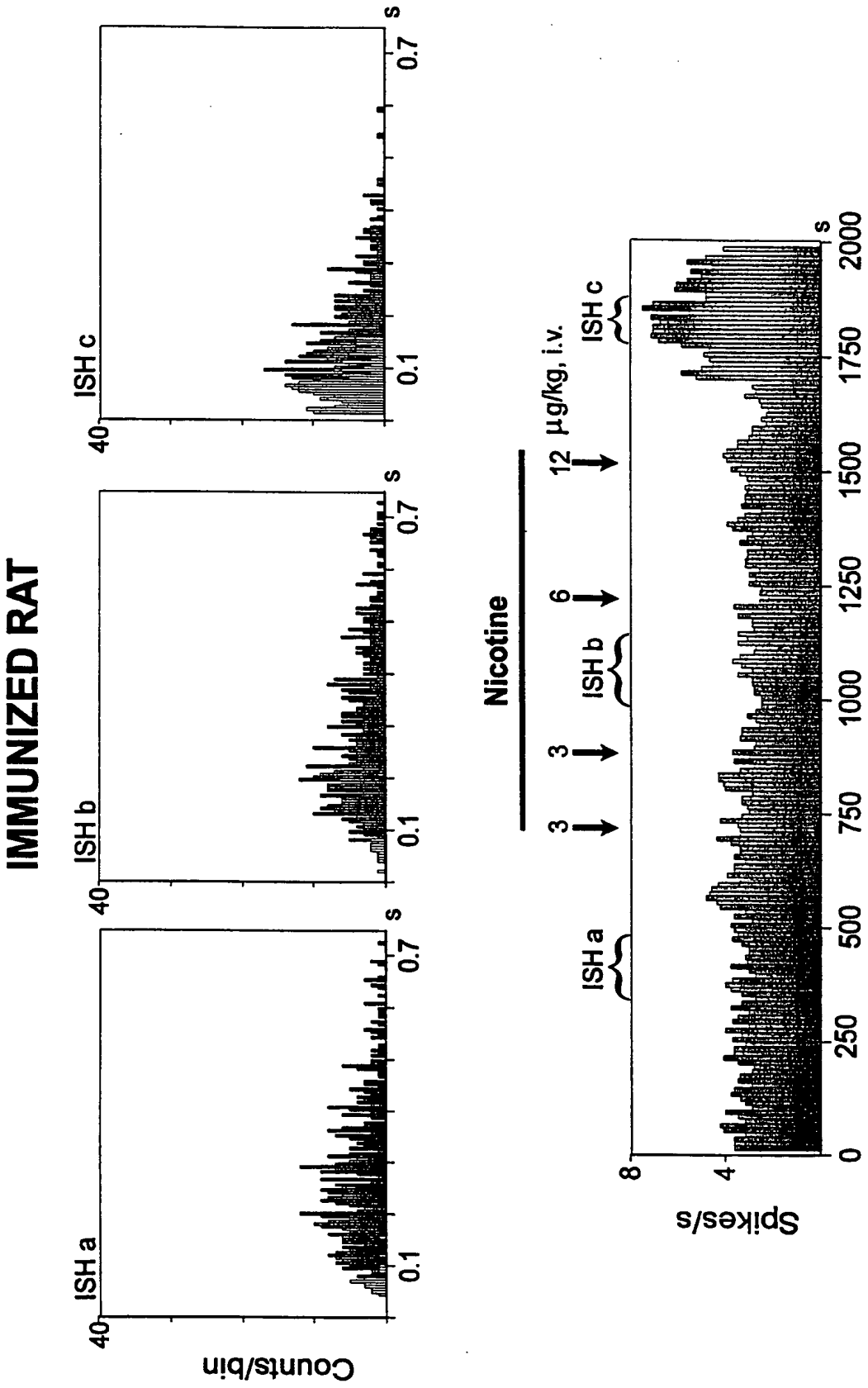

IMMUNIZED RAT
ISH a
ISH b
ISH c
Counts/bin
40
0.1
0.7
s
Nicotine
3
3
6
12
µg/kg, i.v.
Spikes/s
8
4
0
250
500
750
1000
1250
1500
1750
2000

Fig. 6

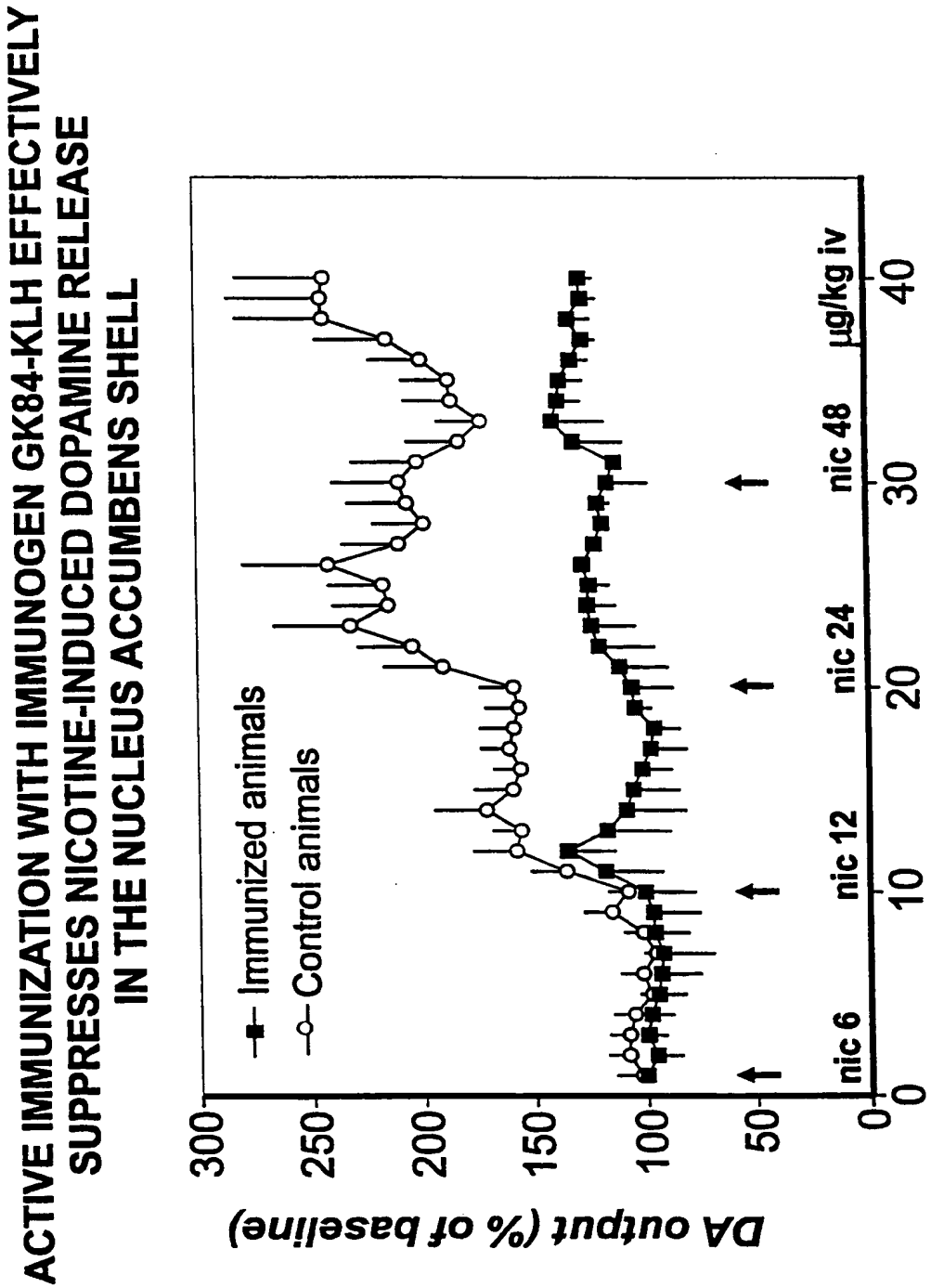

ACTIVE IMMUNIZATION WITH IMMUNOGEN GK84-KLH EFFECTIVELY SUPPRESSES NICOTINE-INDUCED DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS SHELL
Immunized animals
Control animals
DA output (% of baseline)
300
250
200
150
100
50
0
nic 6
nic 12
nic 24
nic 48
µg/kg iv
0
10
20
30
40
Time (min)

Fig. 7a

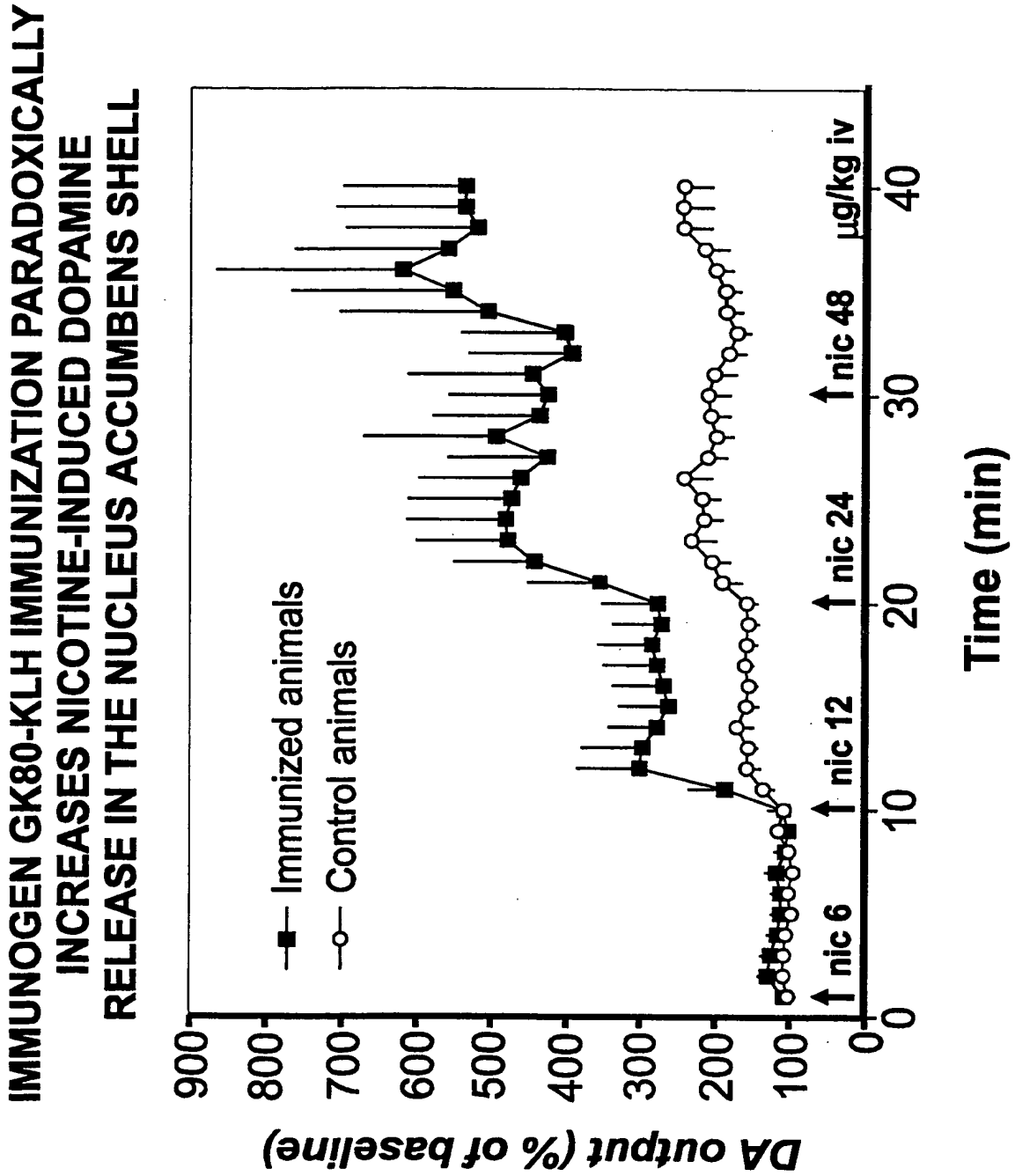

IMMUNOGEN GK80-KLH IMMUNIZATION PARADOXICALLY INCREASES NICOTINE-INDUCED DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS SHELL
DA output (% of baseline)
900
800
700
600
500
400
300
200
100
0
Immunized animals
Control animals
nic 6
nic 12
nic 24
nic 48
µg/kg iv
0
10
20
30
40
Time (min)

Fig. 7b

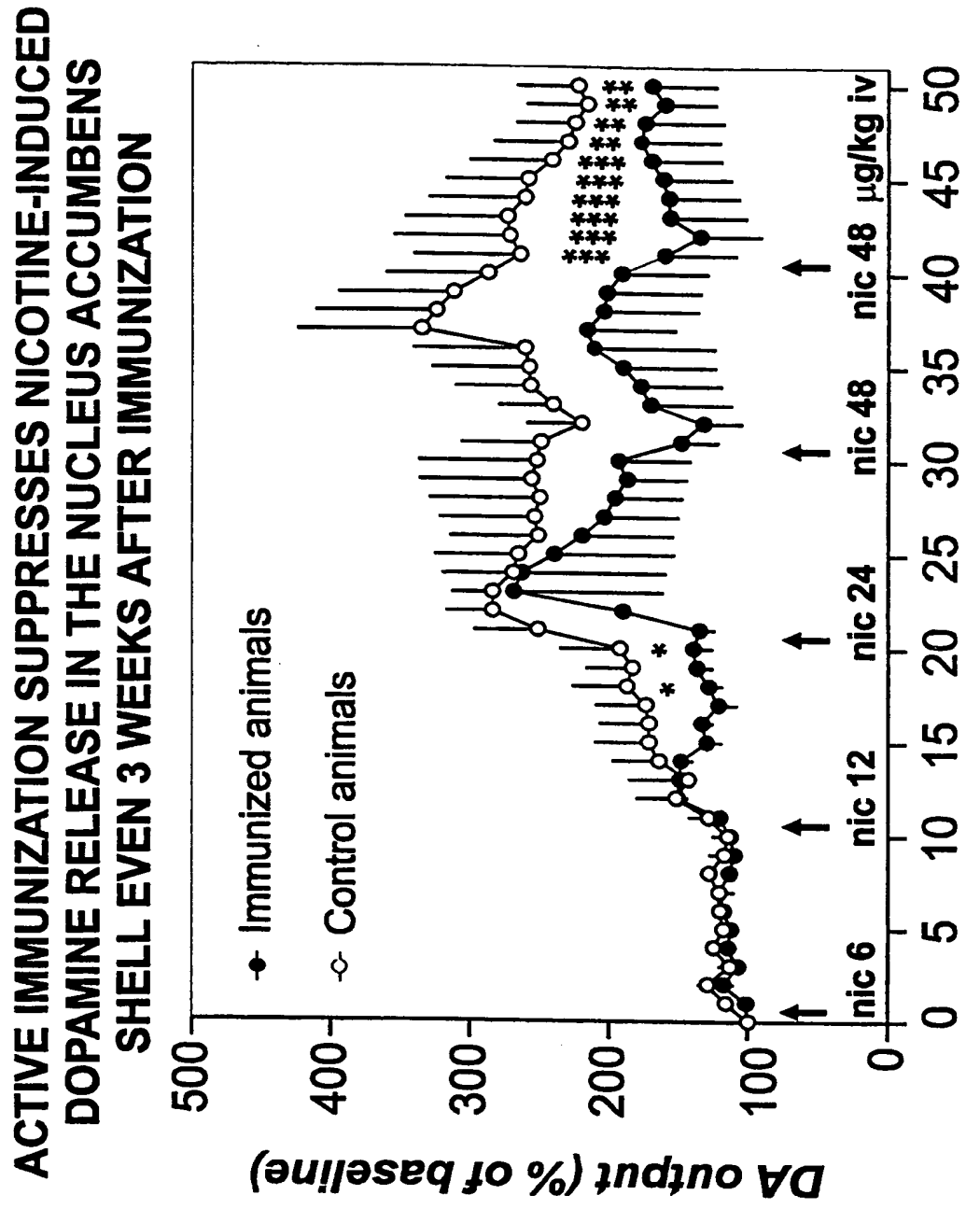

ACTIVE IMMUNIZATION SUPPRESSES NICOTINE-INDUCED DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS SHELL EVEN 3 WEEKS AFTER IMMUNIZATION
DA output (% of baseline)
500
400
300
200
100
0
Immunized animals
Control animals
nic 6
nic 12
nic 24
nic 48
nic 48 µg/kg iv
0 5 10 15 20 25 30 35 40 45 50
Time (min)

Fig. 7c

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/00920

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 39/385
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 9814216 A2 (IMMULOGIC PHARMACEUTICAL CORPORATION), 9 April 1998 (09.04.98), page 5 - page 9; page 27 - page 33, figures 7-10, claim 1, examples 1-3 -- | 1-9 |
| X | The Journal of Pharmacology and Experimental Therapeutics, Volume 283, No 3, 1997, Yoko Hieda et al, "Active Immunization Alters the Plasma Nicotine Concentration in Rats1", page 1076 - page 1081, See especially fig 1 -- | 1-9 |
| A | WO 9630049 A2 (IMMULOGIC PHARMACEUTICAL CORPORATION), 3 October 1996 (03.10.96) -- | 1-9 |

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 14 Sept 1999 | 29 -09- 1999 |
| Name and mailing address of the ISA/<br>Swedish Patent Office<br>Box 5055, S-102 42 STOCKHOLM<br>Facsimile No. +46 8 666 02 86 | Authorized officer<br>Carl-Olof Gustafsson/Els<br>Telephone No. +46 8 782 25 00 |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/00920

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | Research Communication in Chemical Pathology and Pharmacology, Volume 51, No 3, 1986, A. Castro et al, "Nicotine Enzyme Immunoassay" page 393 - page 404<br>-- | 1-9 |
| A | WO 9203163 A1 (CERNY, ERICH, HUGO), 5 March 1992 (05.03.92)<br>--<br>-------- | 1-9 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/00920

Claims 8-9 relate to methods of treatment of the human or animal body by surgery or by therapy/diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

## INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/00920

**Box I** **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 8-9
because they relate to subject matter not required to be searched by this Authority, namely:
see next page

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

**Box II** **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/SE 99/00920

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9814216 A2 | 09/04/98 | AU 6483498 A | 24/04/98 |
| | | US 5876727 A | 02/03/99 |
| WO 9630049 A2 | 03/10/96 | AU 5374996 A | 16/10/96 |
| | | CA 2216658 A | 03/10/96 |
| | | EP 0814843 A | 07/01/98 |
| | | US 5760184 A | 02/06/98 |
| | | US 5773003 A | 30/06/98 |
| | | US 5840307 A | 24/11/98 |
| | | US 5876727 A | 02/03/99 |
| WO 9203163 A1 | 05/03/92 | AT 137977 T | 15/06/96 |
| | | AU 7050091 A | 17/03/92 |
| | | CA 2067205 A | 23/02/92 |
| | | CH 678394 A | 13/09/91 |
| | | DE 59107814 D | 00/00/00 |
| | | EP 0496839 A,B | 05/08/92 |
| | | JP 5502871 T | 20/05/93 |